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Water metabolism of wolves in winter: Effects of varying food intake and exercise

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University of Alaska Fairbanks, 1986

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WATER METABOLISM OF WOLVES IN WINTER: EFFECTS OF VARYING FOOD INTAKE AND EXERCISE

A

THESIS

Presented to the Faculty of the University of Alaska
in Partial Fulfillment of the Requirements
for the Degree of

DOCTOR OF PHILOSOPHY

By
Lee Michael Philo, A.B., V.M.D.
Fairbanks, Alaska
December 1986

WATER METABOLISM OF WOLVES IN WINTER:

EFFECTS OF VARYING FOOD INTAKE AND EXERCISE

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ABSTRACT

The only free water available to wolves during arctic winter is snow. Snow consumption involves an energy cost due to melting the snow and increasing the temperature of the resulting water to deep body temperature. Wolves are subject to negative energy balance when prey availability is inadequate. When negative energy balance is prolonged, the energy cost of snow consumption could shorten the time to death by starvation. It was therefore hypothesized that during negative energy balance in winter, wolves reduce energy expenditure by suppressing snow intake. The goal was to determine whether wolves conserve a significant quantity of energy by suppressing snow intake during negative energy balance in winter.

The hypothesis was tested by varying food intake and exercise of captive wolves during winter in arctic Alaska. Experimental negative energy balance was imposed in three ways: (1) undernutrition, (2) fasting and (3) forced exercise on a treadmill with no change in food intake.

Results of testing the hypothesis varied among experiments, but overall the findings refuted the hypothesis. When the wolves were undernourished, there was indirect evidence of suppressed snow intake. When the wolves were fasted, there was indirect evidence of enhanced snow intake. When the wolves were exercised with no change in food intake, there was indirect evidence of both suppressed and enhanced snow intake, but the evidence of enhancement was more conclusive. The indirect

evidence of enhanced snow intake during either fasting or the exercise trial was sufficient to refute the hypothesis.

The wolves did not conserve a significant amount of energy by suppressing snow intake. When snow intake was suppressed during undernutrition, less than 1% of the calculated daily energy expenditure was saved. There was no unequivocal evidence of snow intake suppression in any other experiment. It is concluded that when energy balance is negative during winter, wolves do not suppress snow intake to conserve energy.

DEDICATION

This thesis is dedicated to three of my best friends:

Barbara D. Jackson

Arthur B. Callahan

Thomas F. Albert

They made it possible.

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ABBREVIATIONS

ADH	Antidiuretic Hormone
ANOVA	Analysis of Variance
BUN	Blood Urea Nitrogen, in mg · dl^{-1}
BW	Body Weight, in kg
$\underline{BW}$	Mean Body Weight, in kg, for a trial
BW_i	Initial Body Weight, in kg, for a trial
BW_f	Final Body Weight, in kg, for a trial
ΔBW	Change in Body Weight, in kg, for a trial
CPM	Counts Per Minute
d	Day
D	Number of Days in a trial
DMI	Dry Matter Intake, in g · kg^{-1}
DPM	Disintegrations Per Minute
ECF	Extracellular Fluid Space, in l
ECF%	Extracellular Fluid Content, in % of body weight
ECF % of Lean	Extracellular Fluid Content, in % of lean body weight
ED	Gross Energy Digestibility
EFF	Counting Efficiency of a scintillation counter, expressed as a decimal
ESR	External Standard Ratio
%Fat	Body Fat, in percent of body weight
F_i	Initial Body Fat Fraction, in kg, for a trial

F_f	Final Body Fat Fraction, in kg, for a trial
ΔF	Change in Body Fat, in kg, for a trial
k	Daily Fractional Water Turnover Rate
ICF	Intracellular Fluid Volume, in l
ICF%	Intracellular Fluid Content, in % of body weight
ICF % of Lean	Intracellular Fluid Content, in % of lean body weight
ISF	Interstitial Fluid Volume, in l
ISF%	Interstitial Fluid Content, in % of body weight
ISF % of Lean	Interstitial Fluid Content, in % of lean body weight
ΔLean	Change in Lean Body Mass, in kg, during a trial
Nbal	Nitrogen Balance, in $mg \cdot kg^{-1} \cdot d^{-1}$
ΔO	Change in Nonfat Nonprotein Body Solids, in kg, during a trial
ΔP	Change in Body Protein, in kg, during a trial
PV	Plasma Volume, in l
PV%	Plasma Volume Content, in % of body weight
PV % of Lean	Plasma Volume Content, in % of lean body weight
RIHSA	Radioiodinated (^{125}I) Human Serum Albumin
SG	Specific Gravity
TBW	Total Body Water, in l
ΔTBW	Change in Body Water, in l, during a trial
TBW%	Body Water Content, in % of body weight

TCA	Trichloroacetic Acid
TOH	Tritiated Water
t_0	Time Zero
$t_{1/2}$	Biological Half Life of a Radioisotope, in d
WI	Water Intake, in ml $\cdot$ kg^{-1} $\cdot$ d^{-1}
WO	Water Output, in ml $\cdot$ kg^{-1} $\cdot$ d^{-1}
WTR	Water Transfer Rate, in ml $\cdot$ kg^{-1} $\cdot$ d^{-1}
UW	Urine Water Content, in %

EXPLANATION OF TERMS

Enhanced Fecal Water Loss: increased water loss via the gut due to increased fecal water content. This increased water loss occurred in the experimental animals (wolves) during decreased total daily fecal water loss associated with fasting. It also occurred when total daily fecal water loss increased with increased daily exercise (running on a treadmill).

Enhanced Snow Intake: the increased snow intake associated with decreased urine osmolality and increased fecal water content (when tissue hydration did not decline). This increased snow intake occurred in the experimental animals (wolves) during fasting and presumably decreased the amount by which snow intake declined due to decreased food intake. This increased snow intake also occurred with increased daily exercise (running on a treadmill, no change in food intake) and was in addition to that compensating for increased evaporative water loss.

Enhanced Urinary Water Loss: increased water loss via the kidneys due to decreased urine osmolality. This increased loss occurred in the experimental animals (wolves) during decreased total daily urine water loss associated with fasting. It also occurred when total daily urine water loss was unchanged after increased daily exercise (running on a treadmill).

Free Water Intake: ingestion of liquid water or snow, not including metabolic water or water in food.

Preformed Water: water in the food.

Overall: refers to changes or processes occurring over an entire trial.

Quench: in liquid scintillation counting, reduction in the luminous output of a radioactive sample due to interference with energy transfer at some point between beta decay of atoms and excitation of both photocathodes of the detector.

Suppressed Snow Intake: The further reduction of snow intake beyond that associated with reduced food intake. Suppression of snow intake was determined from indirect evidence (dehydration, decreased fecal water content, decreased proportion of snow in total daily water intake).

Water Intakes: water entering the body water pool, namely snow preformed water and metabolic water.

Water Outputs: water leaving the body water pool, namely urinary, fecal and evaporative water.

% of Lean: method of expressing body water subcompartments relative to fat-free body mass.

ACKNOWLEDGMENTS

I am grateful to all the Naval Arctic Research Laboratory staff who ensured the successful completion of this project. Craig George and Beth Meininger helped me weigh wolves, move cages, take blood samples and inject isotopes. These tasks were more difficult than they sound, particularly at - 30C, and I am grateful for the dedication and skill exhibited by Craig and Beth. Sally A. Manning and Debbie Eberhardt performed all on-site clinical laboratory analyses, and I greatly appreciate their diligence. Pat Reynolds and George Selby, successive Animal Research Facility supervisors, scheduled all A.R.F. activities and staff assignments around my experiments.

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GENERAL INTRODUCTION

During the arctic winter, air temperature is often -50C or lower, and sunlight is absent for approximately two months. Snow cover is present as early as September and often persists into June. Arctic terrestrial mammals have adapted to these conditions for year-round survival, though the adaptations are not necessarily unique to the Arctic. Such adaptations can be grouped as behavioral, morphological or physiological.

Behavioral adaptations aid many mammalian species in living year-round in the Arctic. For example, postural variations such as curling can decrease the rate of body heat loss by reducing the exposed surface area (Irving 1972). Migration between summer and winter ranges by caribou (*Rangifer tarandus*) is a behavior that precludes depletion of local forage (Irving 1972) and reduces exposure to parasites and predators. The social behavior of wolves (*Canis lupus*) is a major factor in their success in hunting caribou, raising pups and protecting territory. Small mammals, such as lemmings (*Dicrostonyx, Lemmus*) and arctic ground squirrels (*Citellus undulatus*), conserve heat in winter through behaviors such as tunneling and nest building (Irving 1972).

Examples of morphological adaptations include: (1) increased haircoat insulation during the winter in arctic foxes (*Alopex lagopus*), caribou and wolves and (2) antlers in caribou which facilitate courtship, fighting and perhaps migration (Bubenik 1975).

There are numerous examples of physiological adaptations that aid mammals in living year-round in the Arctic. Breeding is timed so that

young are born in the spring, when conditions are most favorable for survival ("anticipatory preparation", Irving 1972). Regional heterothermy in wolves and arctic foxes allows the maintenance of foot temperatures near 0C, thereby minimizing heat loss (Irving 1972, Henshaw *et al.* 1972). Reducing metabolic rate during winter conserves energy in large mammals such as caribou and deer (McEwan and Whitehead 1970). In contrast, small mammals, such as lemmings, weasels (*Mustela rixosa*, *Mustela ermined*) and ground squirrels must either increase heat production or hibernate in winter (Irving 1972, Casey and Casey 1979). The reduced metabolic rate seen in caribou and deer is associated with increased insulation (haircoat), but a comparable increase in fur would immobilize a small mammal.

The wolf is an example of a highly adapted arctic terrestrial mammal and, as such, utilizes behavioral, morphological and physiological adaptations. Some of these adaptations were listed above.

Like other large arctic adapted species the wolf must deal with snow for approximately nine months of the year. This snow cover in the Arctic provides both advantages and disadvantages to wolves and is a factor in hunting success and ultimately survival. The hard-packed snow of the tundra may facilitate travel and the chasing of prey, while the softer, deeper snow in forested areas usually restricts travel to trails and impedes the chase (Irving 1972, Mech 1970). Snow may provide shelter and act as a wind-break. The snow layer also provides the microhabitat for small mammals which may be a food resource when larger prey are not available.

One of the most important aspects of snow in the life history of wolves is that, during winter, snow is the only nonmetabolic water source other than the preformed water in prey animal tissue. Consuming snow carries an energy cost in melting the snow and raising the temperature of the resulting water to body temperature. Based on the heat of fusion of water and specific heats of water and ice (Weast 1970), every kilogram of snow consumed at -40C costs 132 kilocalories. This energy cost of consuming snow is expected to have the greatest effect during periods of negative energy balance. During negative energy balance, water is still required to eliminate osmotically active metabolic waste products and to replace evaporative losses. Some of this water comes from the preformed water in prey carcasses and from the metabolic water of oxidized food and body tissues. In the short term, some of this water may come from intracellular and extracellular water compartments. In the long term, an external water source is necessary, with snow being the only readily available source. Thus, consuming snow throughout a protracted period of food shortage could shorten the time to death by starvation.

Arctic wolves are subject to negative energy balance during winter if prey availability is inadequate. Wolves have adapted to changing food availability by gorging when a kill is made and fasting between kills (Mech 1970). While wolves have adapted to short-term variation in prey availability, they may starve to death during prolonged food shortage. Those most likely to starve are lone wolves and pack subordinates (Mech 1970). Lone wolves lack the cooperative pack effort, and pack subordinates are the last to eat when food supply is marginal.

Certain conditions in the life history of the wolf may exacerbate negative energy balance or precipitate the condition when energy balance is marginal. Alternate gorging and fasting may contribute to negative energy balance because of the inefficiency in alternating catabolism and anabolism of body energy stores. In addition, exercise associated with hunting and social interactions contributes to negative energy balance if these activities continue when food supply is marginal.

Even though snow is the only source of nonprey water readily available during arctic winter, the relationship between snow consumption and negative energy balance in the wolf is poorly understood. There is little in the literature which can directly address this issue. No experimental work in wolves and very little in dogs has been done regarding the relationship between water metabolism and energetics. Most of our basic understanding comes from non-canid mammalian studies. In instances where this relationship has been studied, there has been variation as to which aspects of water metabolism and energetics have been measured. Regarding water metabolism, water transfer rate or water intake has been measured, while regarding energetics, investigators have measured energy expenditure, energy intake or dry matter intake.

When considering the relationship between water metabolism and energetics it must be remembered that they are linked through the osmotic activity of dietary components. These osmotically active components affect water transfer rate in two ways. First, osmotic wastes, which result from ingested food, leave the body in the urine and feces and remove water by osmotic drag. Water loss causes loss of extracellular fluid, and,

subsequently, increased plasma osmolality. Second, the osmotic action of absorbed food components further increases plasma osmolality. The hypothalamus detects the increase in plasma osmolality and stimulates thirst (Guyton 1976).

The effect of osmotically active food components on water intake and water transfer rate has been demonstrated in dogs. The water intake of laboratory dogs was directly related to daily energy intake when energy intake ranged from maintenance to 50% above maintenance (Adolph 1939, English and Filippich 1980). Water transfer rate and dry matter intake of laboratory Beagles were directly related when food intake ranged from zero to approximately twice maintenance (Cizek 1959). One would expect the relationship between water transfer rate and dry matter intake to be altered by exposure to cold air and consumption of snow or cold water. However, none of the canine studies included low ambient temperature or snow as the only free water source.

In addition to being linked through the osmotic activity of dietary components, water metabolism and energetics should also be linked through temperature regulation. Since evaporative water loss is a major factor in dissipating the heat of exercise, an increase in daily exercise should increase total daily energy expenditure and thermoregulatory evaporative water loss. In view of this, it seems reasonable to expect a direct relationship between water transfer rate and energy expenditure if food intake remains constant.

The effects of exercise on evaporative water loss have been studied in some canids but not in wolves. Long distance runners, like the African

hunting dog (*Lycaon pictus*) and the domestic dog, eliminate the heat of exercise primarily by evaporation (Taylor *et al.* 1971, Taylor 1974, O'Connor 1975). This exercise-induced water loss is associated with increased free water intake to maintain water balance (Greenleaf *et al.* 1976).

The extent to which water metabolism and energetics remain linked under varying circumstances in animals is unclear. Macfarlane and colleagues (1971) and Kennedy and Macfarlane (1971) found a stoichiometric coupling of water transfer rate and energy expenditure rate in a number of mammalian species. These investigators did not, however, compare the relationship among seasons, food intake levels or exercise levels. Holleman and colleagues (1982a) showed that the water transfer rate-energy expenditure ratio of red-backed voles (*Clethrionomys rutilus*) in a subarctic environment (Fairbanks, Alaska) was significantly lower in winter than in any other season. Thus, in the Arctic, water transfer rate may be relatively lower during winter than during warmer seasons, but the mechanism and selective advantage (if there is one) are not clear. Two possible mechanisms were offered by Holleman and colleagues (1982a): (1) low water intake in winter may be effected by selective foraging on highly digestible, low protein food which minimizes osmotic drag and/or (2) a more efficient nasal countercurrent heat exchanger may reduce respiratory water loss in winter compared to the other seasons. Although these voles seem able to uncouple water metabolism and energetics, there are no data from which to predict whether or not wolves are able to do so.

If wolves are able to uncouple water and energy relationships, they may suppress snow intake when in negative energy balance during winter. Snow intake suppression would decrease the energy expended in melting snow and raising the temperature of that water to body temperature. If wolves suppress snow intake when in negative energy balance, one or more compensatory changes in water metabolism must occur (Figure I.1). Dehydration is one such change. If wolves sustain dehydration while suppressing snow intake, they must have adaptive changes to tolerate dehydration. An adaptive change which enhances tolerance to dehydration is the shifting of fluid among body water subcompartments to conserve intracellular water (Painter *et al.* 1948, Macfarlane *et al.* 1961) and maintain plasma volume (Reese and Haines 1978). This action helps maintain adequate blood volume and therefore cardiac output and blood pressure. A second possible change in water metabolism is enhanced conservation through a decrease in one or more water outputs. For example, urine concentration could increase, fecal water content could decline or respiratory water loss could decline. A third possible change in water metabolism is an altered relative distribution of water intake (into the body water pool) among snow, food water and metabolic water. An increase in metabolic water could compensate for a decrease in snow intake. During negative energy balance, such a compensatory change is possible if metabolism of body energy stores favors fat breakdown since fat provides approximately twice the metabolic water provided by an equal weight of protein or carbohydrate (Van Es 1969). The occurrence of one or more of the three possible changes in water metabolism during negative

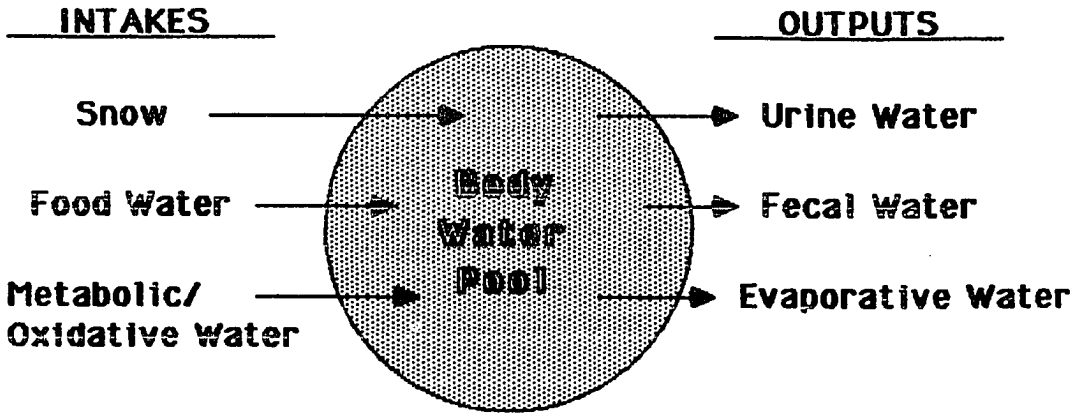

Figure I.1. Body water intakes and outputs of wolves in arctic winter. Increased water conservation may occur through increased urine concentration, decreased fecal water content, decreased evaporative water loss or a combination of these processes. Suppression of snow intake may be brought about by increasing the proportion of water intake in food and/or the proportion of water intake as metabolic water. Dehydration occurs when the size of the total body water pool declines due to water output exceeding intake.

energy balance in winter would be indirect evidence that wolves suppressed snow intake.

The possibility that wolves save energy during periods of negative energy balance by suppressing snow intake was the basis for the present study. I hypothesized that during negative energy balance in winter, wolves reduce energy expenditure by suppressing snow intake. Suppression of snow intake is defined as the further reduction of snow intake beyond that associated with reduced food intake. The overall objective was to test the hypothesis by subjecting captive wolves to various forms of negative energy balance during arctic winter and determining whether snow intake was suppressed. Indirect evidence of suppressed snow intake was provided by measuring water intake (snow, free water in food, metabolic water), water output (urinary, fecal and evaporative water), and degree of dehydration.

The hypothesis was tested by using two groups of captive wolves in three experiments which are detailed in the three chapters of this thesis. In each experiment, wolves were placed in negative energy balance either by manipulating food intake (in two experiments) or by increasing energy expenditure (one experiment). In the first experiment, the objective was to determine the effect of undernutrition on winter water metabolism in wolves. The second experiment had two objectives: (1) to determine the effect of fasting on winter water metabolism in wolves and (2) to determine the overall effect of alternately gorging and fasting on winter water metabolism in wolves . The objective of the third experiment was

to determine the effect of increased daily exercise (with unchanged food intake) on winter water metabolism in wolves.

Chapter 1

Water Metabolism of Captive Wolves in Winter.

I. Effects of Varying Food Intake.

INTRODUCTION

The daily food intake of wild wolves varies substantially above and below the estimated daily requirement. In winter, a 30 - 45 kg adult wolf requires approximately 1.4 to 1.9 kg of meat daily (Mech 1970). Due to variable food availability, the actual food quantity consumed each day varies from zero to several times the requirement. Wolves normally gorge and fast because of short-term variability in food supply, but there is also substantial long-term variability in food intake. When prey are plentiful, wolves consistently consume, on average, two to three times the estimated minimum amount of meat required (Burkholder 1959, Mech 1970, Holleman *et al.* 1979). When prey are sufficiently scarce for weeks or months, mean daily food quantity is inadequate and wolves may starve to death.

Voluntary snow intake and water transfer rate of wild wolves undoubtedly varies with food intake during winter. There is only indirect evidence to support this prediction. During summer, wild wolves drink the most water when food intake is highest (Mech 1970). Presumably, wolves also consume the most snow when food intake is highest during winter. The water-food relationship has been studied in laboratory dogs but not in other canids. Voluntary water consumption and water transfer rate of laboratory dogs varied directly with dry matter intake from zero to at least twice maintenance (Adolph 1939, Cizek 1959, English and Filippich 1980). The effects of low air and water temperatures on the water-food relationships of canids are not known because neither low ambient

temperature nor cold water were variables in the canine studies. Thus, water transfer rate and voluntary snow intake may be directly related to dry matter intake in wolves, but direct measurements remain to be made.

Consuming snow during negative energy balance in winter may uncouple the water transfer rate-dry matter intake and snow intake-dry matter intake relationships. The water-food relationship has not been studied during negative energy balance but has been compared among seasons. Holleman and colleagues (1982a) reported the uncoupling of a similar relationship in red-backed voles. The water transfer rate-energy expenditure ratio of the voles declined during winter compared with summer, but the mechanism and selective advantage were unclear.

There is a possible selective advantage to uncoupling the water-food relationship in wolves: by decreasing the ratio of snow intake to dry matter intake during negative energy balance in winter, wild wolves might conserve enough energy to survive. Water is required for elimination of metabolic wastes and for the evaporative heat loss of exercise, even when food intake is inadequate or absent. Some of this water may be supplied by oxidation of food or body tissues and by ingestion of fresh carcasses. Additional water requirements must be met by consuming snow or frozen carcasses, but such consumption carries an energy cost. Every kilogram of snow or ice consumed at -40C costs 132 kilocalories while melting and warming to deep body temperature. Thus, when wild wolves are undernourished for weeks or months, the energy cost of consuming snow could shorten the time to death by starvation.

There is indirect evidence that sheep (Blaxter *et al.* 1959) and rats (Fregly 1982) suppressed cold water intake at low ambient air temperatures. The sheep had a lower (by 50%) water intake-dry matter intake ratio when cold-stressed and given cold water (below 10C) than when not temperature-stressed and given warm water (28C) (Blaxter *et al.* 1959). When rats were cold-stressed by exposure to 5C air after acclimation to 26C air, the water intake-food intake ratio declined (Fregly 1982). In addition, sheep (Degen and Young 1981) and cattle (Degen and Young 1984) reduced water intake during winter when snow was substituted for fresh water. Since daily food intake by these sheep and cattle was unchanged, the water intake-dry matter intake ratios declined after snow was substituted for fresh water.

The experiment described in this chapter was designed to test the hypothesis that wolves reduce energy expenditure by suppressing snow intake when energy balance is negative during winter. The objective was to determine the effects of undernutrition on winter water metabolism of captive wolves.

METHODS

Experimental Animals

The five wolves used were male siblings born and raised in captivity at the Naval Arctic Research Laboratory in Barrow, Alaska. They were nine years old and weighed 35 to 40 kg at the start of the experiment. The experiment was conducted from mid-January to mid-March. Each wolf was kept in a steel metabolism cage, 0.9 m wide · 1.2 m long · 0.9 m high, inside an unheated building. The wolves were therefore subjected to ambient air temperature (-5 to -33C) but sheltered from wind and precipitation. Artificial light was provided for six to eight hours daily.

Experimental Conditions.

Maintenance food intake was defined as the minimum daily quantity which prevented body weight loss. The food used in this study was Zu/Preem (Hill's Division Riviana Foods, Topeka, KA), a commercial meat-based diet for carnivores. Estimated maintenance Zu/Preem intake during winter for caged adult wolves at the laboratory was 37.50 g · kg^{-1}.

The variable in this experiment was level of food intake. The experiment began with two weeks of conditioning in metabolism cages with the estimated maintenance Zu/Preem intake. Conditioning was followed immediately by three consecutive two-week trials in which the level of Zu/Preem intake differed. The first trial was designated Undernutrition, and Zu/Preem intake was approximately half maintenance, 18.75 g · kg^{-1}. The second trial was designated Maintenance, and

Zu/Preem intake was approximately maintenance, 37.50 g • kg^{-1}. The third trial was designated Overnutrition, and Zu/Preem intake was approximately twice maintenance, 75.00 g • kg^{-1}. The Zu/Preem water content was 56.9 ± 1.4% (n = 82). The respective daily dry matter intakes for Undernutrition, Maintenance and Overnutrition were 8.08, 16.16 and 32.32 g • kg^{-1}. Throughout a trial, each wolf received the same absolute food quantity every day, based on body weight at the start of that trial.

Food and water consumption were measured daily. Preweighed food and snow were given to each wolf once a day in the early afternoon and removed after about two hours. The food was kept in plastic bags to prevent evaporation. Food was placed in an incubator for several hours before feeding so food temperature would approximate the body temperature of a fresh kill. Uneaten snow and food were weighed. Uneaten food was dried and reweighed to determine water content. Rarely did any food remain uneaten, but snow was usually offered in excess of that consumed. When vomiting occurred, the regurgitated food was immediately reoffered.

The experimental day was considered to begin at approximately 1600, following food and snow consumption. As a result, 24 hour urine volumes and fecal weights were measured between feedings. Body weights were determined and blood samples were collected near the end of the experimental day.

Body Weight

Each wolf was weighed to the nearest 0.1 kg every morning. The wolf was placed in a weighing cage, and the weighing cage with wolf was suspended from a spring scale. For comparisons, body weights in each trial were expressed as fractions of body weight on day one of the trial; the actual body weights (in kg) are given in Appendix A.

Body Temperature

Body temperature was monitored via radiotelemetry. A temperature-sensitive radiotransmitter (148 to 149 MHz, model Mark IV, Telonics, Mesa, AZ) was surgically implanted free-floating in the abdomen of each wolf at least three weeks prior to the start of the experiment. The signals were received on a model TR-1 receiver (Telonics) and passed to a model TDP-1 (Telonics) digital processor which displayed pulse period in milliseconds. Pulse period was converted to degrees Centigrade according to a calibration curve prepared for each transmitter prior to implantation. Reception equipment was located in an adjacent building so that the wolves were not disturbed during temperature recording. Temperatures of all five wolves were recorded three times every day: in the morning before any disturbance, in the afternoon immediately before feeding and about two hours after feeding. Twice each week, body temperatures were recorded every hour for 24 hours.

Ambient Temperature

Ambient temperature was recorded with every body temperature measurement. Ambient temperature was measured via a thermocouple wire placed at the same height above the floor as the caged wolves. The wire was connected to a thermocouple model digital thermometer (Omega Engineering, Inc., Stamford, CT).

Blood, Urine, Fecal and Food Analyses

Sample Collection

Blood samples were collected on days 4, 7, 11 and 14 of each trial. For blood collection, each wolf was transferred to a squeeze cage and physically restrained. A heat lamp was used to protect syringe contents and bare hands from the cold. Whole blood was collected for hematocrit with calcium ethylene diaminetetracetate (EDTA) anticoagulant. Serum from clotted blood was collected for electrolyte and total protein concentrations plus osmolality.

Urine drained from a slanted trough beneath each cage into a beaker and was collected every day before feeding. Mineral oil in the beaker prevented evaporation, and a heating pad plus styrofoam insulation around the beaker prevented freezing.

Feces were collected from a tray beneath each cage floor. If feces were trapped between the bars of the cage floor they were dislodged with a long rod passed through the cage sides or top. Most of the daily collection was made between 0600 and 0800 because the wolves defecated the most when first disturbed. Additional feces were collected

when seen. One day per week, feces were collected (if present) every 20 minutes for 24 hours. The feces were placed in Whirl-Pack bags (NASCO), labelled, weighed and frozen.

Eighty-two Zu/Preem samples were collected in Whirl-Pak bags during two winters. All samples were kept frozen in sealed Whirl-Pak bags until water content was measured.

Water Content Analyses

Urine froze to the plastic liner of the trough beneath each cage. Urine also froze to weighing and squeeze cage floors when wolves urinated in these cages. This frozen urine was scraped free, weighed, dried and reweighed to calculate water content.

The urine in beakers was allowed to warm to approximately room temperature, and volume was measured. A 100 ml sample was transferred to a volumetric flask, weighed, dried and reweighed to determine water and solids contents. Specific gravity was measured with a hydrometer.

After the experiment, known volumes of wolf urine were poured through metabolism cages. The quantity recovered in the beakers and plastic liners was measured and a correction factor was calculated. This factor accounted for urine lost to collection, i.e. evaporated from the plastic liner and frozen to the cage floor. Only 4 ± 2% of the urine poured through the cages was lost.

After the experiment, feces were thawed and dried to constant weight for determination of water and dry matter contents. Zu/Preem samples were dried to constant weight for water content determination.

Hematocrit

Hematocrit was measured with a microhematocrit centrifuge and reader.

Chemical Analyses

Serum from clotted blood was analyzed colorimetrically for total protein, using a clinical spectrophotometer (Serometer 360, Mallincrodt, Inc., Bohemia, NY).

Sodium and potassium concentrations in serum and urine were determined with a flame photometer (Model IL 143, Instrumentation Laboratories, Inc., Lexington, MA). Serum and urine chloride concentrations were determined colorimetrically with a narrow-band spectrophotometer (Model DU-2, Beckman Instruments, Inc., Fullerton, CA). Serum and urine osmolalities were determined by the freezing point depression method using an osmometer (Model 3D II, Advanced Instruments, Inc., Needham Heights, MA). Fecal samples from days 4, 7, 11 and 14 and three dried food samples were used for electrolyte determination. Electrolyte analyses of food and feces were performed at the Agricultural Experiment Station, Palmer, AK. Zu/Preem and fecal sodium and potassium were determined with an atomic absorption spectrophotometer (sodium by flame emission, potassium by straight aspiration) (Model 5000, Perkin-Elmer). Urine and fecal electrolyte concentrations were multiplied by 24 hour urine volumes and 24 hour dry fecal weights to give total daily losses. Food electrolyte concentration was multiplied by total daily dry food consumption to give daily electrolyte consumption.

Urine and fecal nitrogen analyses were performed on samples collected during days 4, 7, 11 and 14 of each trial. Three food samples were analyzed for nitrogen content. Concentration of urine nitrogen as ammonium was measured by an automated procedure (Technicon Industrial Method No. 98-70W, Technicon Industrial systems, Tarrytown, NY) on an autoanalyzer (Autoanalyzer II, Technicon). Total urine nitrogen concentration (ammonia plus urea nitrogen) was measured colorimetrically on a narrow-band spectrophotometer (Lambda 1, Perkin-Elmer, Tukwila, WA). Ammonia nitrogen concentration was subtracted from total urine nitrogen concentration to give urea nitrogen concentration. Dried food and fecal samples were used for nitrogen determination. Nitrogen analyses of food and feces were performed at the Agricultural Experiment Station, Palmer, AK. Nitrogen concentration was determined by an automated procedure (Pace and Rathbun 1945) with an autoanalyzer (Autoanalyzer II, Technicon). Urine and fecal nitrogen concentrations were multiplied by 24 hour urine volumes and 24 hour dry fecal weights to give total daily losses. Food nitrogen concentration was multiplied by total daily dry food consumption to give daily nitrogen consumption.

Urine energy concentration was calculated for days 4, 7, 11 and 14 of each trial, and fecal energy concentration was measured for day 14 of each trial. Two food samples were used. Urine energy concentration was calculated from urea concentration since urinary energy in carnivores is present primarily as the chemical energy of urea (Kleiber 1975). Urea nitrogen concentration ($mg \cdot ml^{-1}$) was multiplied by 5.41, the urea energy

content (cal • mg^{-1}) of urea nitrogen, to estimate urine energy concentration (cal • ml^{-1}). Dried food and fecal samples for energy concentration were ground through a number 40 mesh screen in a Wiley mill. Gross energy concentration was determined with an adiabatic bomb calorimeter (Parr Instrument Co., Inc., Moline, IL). Urine and fecal energy concentrations were multiplied by 24 hour urine volumes and 24 hour dry fecal weights to give total daily losses. Food energy concentration was multiplied by total daily dry food consumption to give daily energy consumption.

Total Body Water, Water Transfer Rate and Body Fat

Total body water (TBW) and water transfer rate (WTR) were determined with tritiated water (TOH). On day one of each trial, each wolf was injected by venapuncture (cephalic vein) with 450 µCi TOH. The injection syringe was flushed with approximately 20 ml of normal saline, and the saline was injected intravenously. Blood samples were collected from the opposite cephalic vein three hours post-injection and on days 4, 7, 11 and 14.

Samples were processed as follows. Equal volumes (1.0 ml) of plasma and 20% trichloroacetic acid (TCA) were mixed well and centrifuged to precipitate all plasma proteins. One milliliter of supernatant was mixed with 10.0 ml scintillation cocktail (Aquasol II, New England Nuclear Corp., Billerica, MA) and 0.25 ml glacial acetic acid in a scintillation vial, and the mixture was counted in a liquid scintillation counter (Model 6892, Tracor, Inc., Elk Grove Village, IL). Disintegrations per minute (DPM) were

calculated as net counts per minute (CPM) corrected for counting efficiency. Counting efficiency was measured with quenched tritium standards, using the external standard ratio technique. Total body water was calculated from the formula:

$$TBW = V_1 \cdot V_2 \cdot DPM_I \cdot F_1 \cdot V_3^{-1} \cdot DPM_S^{-1} \cdot F_2^{-1} \qquad (1.1),$$

where

TBW = total body water in ml

DPM_I = disintegrations per minute in the injection stock solution

DPM_S = disintegrations per minute in the sample after extrapolation to time zero

V_1 = volume injected in ml

V_2 = volume of plasma counted in ml

V_3 = volume of injection stock solution counted in ml

F_1 = correction factor for the water content of protein = 0.93 (Ryan *et al.* 1956, Bauer *et al.* 1975a, Bauer *et al.* 1975b)

F_2 = correction factor for DPM of sample due to change in liquid volume after plasma protein precipitation with 20% TCA = 0.97.

F_2 was computed by first determining DPM in 1.0 ml supernatant after precipitation (theoretically equivalent to 0.5 ml plasma). In a duplicate sample, 0.5 ml plasma was mixed with 0.5 ml 20% TCA. The entire supernatant was counted. The precipitate was washed repeatedly until wash counts were below background. F_2 is the ratio of DPM of the first sample to DPM of the duplicate sample.

For WTR determination, $\log_{10}$ sample DPM was plotted against time (days). Biological half life ($t_{1/2}$, d) of TOH was calculated from the slope of this plot. The daily fractional turnover rate (k, d^{-1}) of body water was then calculated as:

$$k = 0.693 \cdot t_{1/2}^{-1} \quad \text{(Cameron and Luick 1972)} \qquad (1.2),$$

and water transfer rate (WTR, ml $\cdot$ kg^{-1} $\cdot$ d^{-1}) was calculated as:

$$WTR = k \cdot TBW \quad \text{(Cameron and Luick 1972)} \qquad (1.3).$$

Since a nonsteady state existed during the beginning of each trial, water outflow and inflow rates were calculated according to equations derived for subjects in which TBW was changing with rapid growth (Holleman *et al.* 1982b). The complete analysis and results are in Appendix B. A discussion of the TOH technique and assumptions made in this study appears in Appendix C.

Body fat was calculated from the formula of Pace and Rathbun (1945):

$$\%Fat = 100 - \frac{\%Water}{0.732} \qquad (1.4).$$

Metabolic Water Production and Evaporative Water Loss

There were three water intakes and three water outputs (Figure 1.1). Only four of these were measured directly: water intake in snow and food, and water loss in urine and feces. Metabolic water production and evaporative water loss were measured indirectly.

Water produced metabolically was estimated by two methods. The first estimation, designated Metabolic Water Production, was effected by subtraction of snow and food water intakes from WTR. The second estimate, designated Oxidative Water, was the algebraic sum of the

oxidation water for fat, protein and carbohydrate catabolized or added to body stores. Calculation of oxidative water is explained in detail below.

Evaporative water loss was calculated by subtraction of urine and fecal water from WTR. The calculated evaporative water loss includes water lost to salivation when wolves were in squeeze cages. It was not possible to collect the lost saliva.

Calculations

Total change in body protein during a trial (ΔP, kg) was calculated from mean nitrogen balance (Nbal, mg $\cdot$ kg^{-1} $\cdot$ d^{-1}), number of days (D) and mean body weight ($\underline{BW}$, kg) according to Equations 1.5 and 1.6:

$$\underline{BW} = (BW_{initial} + BW_{final}) \cdot 0.5 \quad (1.5)$$

$$\Delta P = Nbal \cdot D \cdot \underline{BW} \cdot (6.25 \cdot 10^{-6} \text{ kg protein} \cdot \text{mg N}^{-1}) \quad (1.6).$$

Total change in body fat during a trial (ΔF, kg) was calculated from initial and final BW (BW_i and BW_f) and body fat fraction (F_i and F_f, from Equation 1.4) according to Equation 1.7:

$$\Delta F = BW_f \cdot F_f - BW_i \cdot F_i \quad (1.7).$$

Total change in nonfat nonprotein dry solids (ash, carbohydrates) during a trial (ΔO, kg) was calculated from ΔP, ΔF, change in TBW (ΔTBW, l) and change in BW (ΔBW, kg) according to Equation 1.8:

$$\Delta O = \Delta BW - \Delta TBW - \Delta F - \Delta P \quad (1.8).$$

Total change in lean wet weight during a trial (ΔLean, kg) was calculated from ΔBW and ΔF according to Equation 1.9:

$$\Delta Lean = \Delta BW - \Delta F \quad (1.9).$$

The ratios ΔTBW/ΔLean and ΔTBW/ΔP were used to evaluate tissue hydration status. Calculated ΔTBW/ΔLean ratios greater than 0.732 indicated dehydration when both terms were negative and increased hydration when both terms were positive. If the two signs differed, negative ΔTBW indicated dehydration and positive ΔTBW indicated increased hydration. The second ratio had an advantage over the first in that the numerator and denominator, ΔTBW and ΔP, were determined independently. A disadvantage of the second ratio was that the water content of protein was more difficult to estimate than was the water content of lean tissue. Water content of wet protein was taken as 73.2%. Calculated ΔTBW/ΔP ratios greater than 2.7 indicated dehydration when both terms were negative and increased hydration when both terms were positive. If the two signs differed, negative ΔTBW indicated dehydration and positive ΔTBW indicated increased hydration.

Oxidative Water was calculated from the yields of metabolized protein, fat and carbohydrate as follows. Food digestibility was estimated as gross energy digestibility (ED) which was calculated from Equation 1.10:

$$ED = (\text{Energy intake} - \text{fecal Energy}) \cdot (\text{Energy intake})^{-1} \quad (1.10).$$

The approximate protein, fat and carbohydrate fractions in the dry food were 0.432, 0.405 and 0.014, respectively. The daily digested intakes ($g \cdot kg^{-1}$) of the the three components were calculated according to Equations 1.11, 1.12 and 1.13:

$$\text{digested protein} = 0.432 \cdot DMI \cdot ED \quad (1.11)$$

$$\text{digested fat} = 0.405 \cdot DMI \cdot ED \quad (1.12)$$

digested carbohydrate = 0.014 • DMI • ED (1.13),

where DMI is daily dry matter intake ($g \cdot kg^{-1}$). These three quantities were converted to their oxidative water equivalents by the factors 0.420, 1.065 and 0.556 $ml \cdot g^{-1}$ for protein, fat and carbohydrate, respectively (Van Es 1969). The results were summed to estimate total oxidative water resulting from food. Two adjustments were made to this total. Oxidative water from catabolism of body protein and fat was added, and oxidative water equivalent to increases in body protein and fat was subtracted. The terms ΔF and ΔP were used to estimate changes in body protein and fat.

Total daily energy expenditure was calculated by first subtracting daily fecal energy and urinary energy from energy intake to give metabolizable energy. To this was added the algebraic sum of the energy equivalents for body protein ($4.4\ kcal \cdot g^{-1}$) and body fat ($9.4\ kcal \cdot g^{-1}$) (Maynard *et al.* 1979) gained or lost. Individual energy expenditure data are listed in Appendix D.

The water transfer rate-energy expenditure ratio was calculated after converting the water and energy units to moles. In converting energy expenditure from kcal to moles (Brody 1945), an estimated respiratory quotient of 0.71 was used for Undernutrition, and an estimated respiratory quotient of 0.82 was used for Maintenance and Overnutrition.

Daily sodium, potassium and nitrogen balances were calculated by subtracting fecal and urine values from intake. Energy balance was calculated by subtracting total daily energy expenditure from total daily

metabolizable energy; the result was numerically equal to the total energy gained by or lost from body tissues.

The zero balance point was the food intake level at which sodium, potassium, nitrogen or energy balance was zero. The zero balance points were calculated from the linear equations of dry matter intake regressed against balances. The calculation was made by setting balance to zero and solving for dry matter intake.

Statistical Analyses

Linear regression and correlation analysis was used to describe three sets of relationships: water intakes and outputs with dry matter intake and loss; WTR with various WTR determinants; balances of electrolytes, nitrogen and energy with dry matter intake.

Statistical comparisons among the three trials were made by analysis of variance (ANOVA) with $P = 0.05$. The independent variables were trial, wolf and day. Where first order (two-way) interaction was significant, the analysis was repeated but partitioned by wolf or day as appropriate. Where ANOVA revealed significant differences among trials, the least significant difference ($P = 0.05$) was used to compare trial means in pairs.

RESULTS

Body Weight

The wolves lost weight during Undernutrition and gained weight during Maintenance and Overnutrition (Figure 1.1). The overall rate of gain for Overnutrition was significantly greater than for Maintenance (t-test), but the first significant difference between the two weight-time curves did not occur until day five. By comparison, the Undernutrition curve differed from the Overnutrition curve by day two and from the Maintenance curve by day three.

Water Intakes and Outputs

Mean daily water intakes in food and snow increased with food intake, but calculated metabolic water production did not (Tables 1.1 and 1.2). The relationship between snow intake and dry matter intake was strongly linear (Table 1.3).

Mean daily fecal, urine and evaporative water losses increased with increased food intake (Table 1.1). Fecal, urine and evaporative water losses were linearly related to dry matter intake (Table 1.3). Daily urine water loss was linearly related to daily urine sodium, potassium, chloride and nitrogen losses. Daily fecal water loss was linearly related to daily fecal sodium, potassium, nitrogen and energy losses (Table 1.3).

Fecal water content also increased with increased food intake (Table 1.1). Analysis of variance was not used because there were significant first order interactions between trial and wolf and between trial and day.

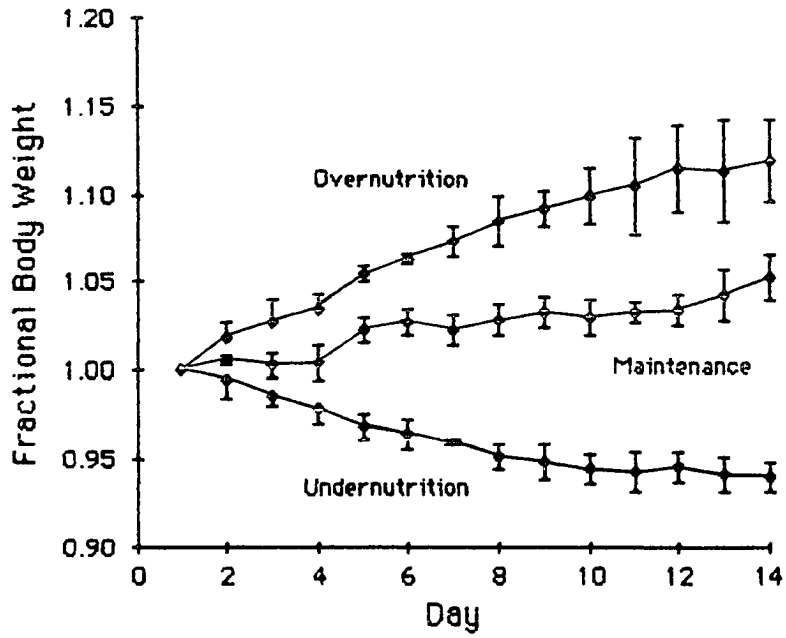

Figure 1.1. Fractional body weights of captive wolves at three food intake levels. Daily food intakes were 18.75 g·kg^{-1} during Undernutrition, 37.50 g·kg^{-1} during Maintenance and 75.00 g·kg^{-1} during Overnutrition. Weights are expressed as fractions of weight on day 1 of each trial. Error bars indicate standard deviation.

TABLE 1.1. Water intake and output parameters of captive wolves at three levels of food intake[a] during winter.

Parameter	Trial		
	Undernutrition	Maintenance	Overnutrition
Water Transfer Rate ($ml \cdot kg^{-1} \cdot d^{-1}$)	25.0 ± 5.6 (5)	39.7 ± 6.7 (5)	66.1 ± 10.5 (5)
Fractional Daily Turnover Rate (d^{-1})	0.043 ± 0.010 (5)	0.068 ± 0.011 (5)	0.114 ± 0.020 (5)
Water Intake ($ml \cdot kg^{-1} \cdot d^{-1}$)			
Snow Water	7.9 ± 7.2 (69)	12.6 ± 9.2 (70)	22.6 ± 14.0 (69)
Food Water	11.1 ± 0.2 (69)	20.9 ± 0.3 (70)	37.9 ± 6.1 (69)
Metabolic Water[b]	6.0 ± 4.5 (69)	6.2 ± 6.5 (70)	5.7 ± 11.1 (69)
Oxidative Water[b]	6.6 ± 0.7 (5)	7.6 ± 3.1 (5)	10.1 ± 1.8 (5)
Water Output ($ml \cdot kg^{-1} \cdot d^{-1}$)			
Urine Water	13.3 ± 6.9 (70)	18.6 ± 8.0 (70)	31.0 ± 13.9 (65)
Fecal Water	2.7 ± 1.0 (70)	5.7 ± 1.9 (70)	14.0 ± 3.8 (65)
Evaporative Water[b]	9.0 ± 4.1 (70)	15.4 ± 4.0 (70)	21.3 ± 8.2 (65)
Fecal Water Content (%)	58.1 ± 3.6 (70)	62.1 ± 5.3 (70)	66.7 ± 2.9 (70)

[a]Undernutrition food intake = 18.75 $g \cdot kg^{-1} \cdot d^{-1}$; Maintenance food intake = 37.50 $g \cdot kg^{-1} \cdot d^{-1}$; Overnutrition food intake = 75.00 $g \cdot kg^{-1} \cdot d^{-1}$. Each trial lasted 14 days. [b]Metabolic water and evaporative water were calculated by subtraction; oxidative water was calculated from the algebraic sum of oxidative water equivalents of nutrients catabolized and body tissue changes. Data are given as mean ± standard deviation (sample size). Results of statistical comparisons are in Table 1.2.

TABLE 1.2. Statistical comparison of body water intake and output parameters, by analysis of variance among trials, for captive wolves at three food intake levels[a] during winter.

Parameter	Trials Compared: Undernutrition & Maintenance	Undernutrition & Overnutrition	Maintenance & Overnutrition
Water Intake			
Snow Water	NS(3)	*	*
Metabolic Water	NS	NS	NS
Oxidative Water	NS	NS	NS
Water Output			
Urine Water	*	*	*
Fecal Water	*	*	*
Evaporative Water	*	*	*(3)
Water Transfer Rate	*	*	*
Fractional Turnover Rate	*	*	*

[a]Undernutrition food intake = 18.75 $g \cdot kg^{-1} \cdot d^{-1}$; Maintenance food intake = 37.50 $g \cdot kg^{-1} \cdot d^{-1}$; Overnutrition food intake = 75.00 $g \cdot kg^{-1} \cdot d^{-1}$. Each trial lasted 14 days. Asterisk (*) indicates statistical significance at P = 0.05; NS indicates lack of statistical significance at P = 0.05. If statistical significance differed among the five wolves, results for majority are shown, followed by number of wolves in parentheses. Data compared in this table are listed in Table 1.1.

TABLE 1.3. Linear regression and correlation of water intakes and outputs with dry matter intake and electrolyte, nitrogen and energy losses of captive wolves at three levels of food intake[a] during winter.

Dependent Variable[b]	Independent Variable[c]	Slope	Y-Intercept	Corr. Coef.	Sample Size
Water Intake in Snow	Dry Matter Intake	0.61	2.9	0.506	208
Water Loss in Feces	Dry Matter Intake	0.46	- 1.4	0.910	210
Water Loss in Urine	Dry Matter Intake	0.74	7.1	0.596	205
Evaporative Water Loss	Dry Matter Intake	0.49	6.1	0.646	205
Water Loss in Feces	Daily Fecal Sodium Loss	0.30	2.9	0.771	53
Water Loss in Feces	Daily Fecal Potassium Loss	0.75	2.1	0.770	53
Water Loss in Feces	Daily Fecal Nitrogen Loss	0.04	3.1	0.746	53
Water Loss in Feces	Daily Fecal Energy Loss	0.19	4.1	0.433	53
Water Loss in Urine	Daily Urine Sodium Loss	0.37	2.3	0.680	58
Water Loss in Urine	Daily Urine Potassium Loss	0.27	- 0.2	0.749	58
Water Loss in Urine	Daily Urine Chloride Loss	0.11	3.7	0.673	58
Water Loss in Urine	Daily Urine Nitrogen Loss	0.02	5.3	0.561	58

[a]Levels of food intake were 18.75 $g \cdot kg^{-1} \cdot d^{-1}$, 37.50 $g \cdot kg^{-1} \cdot d^{-1}$ and 75.00 $g \cdot kg^{-1} \cdot d^{-1}$. [b]Water intakes and outputs in $ml \cdot kg^{-1} \cdot d^{-1}$. [c]Dry matter intake expressed in $g \cdot kg^{-1} \cdot d^{-1}$; urine and fecal electrolyte and nitrogen losses expressed in $mg \cdot kg^{-1} \cdot d^{-1}$; fecal energy loss expressed in $kcal \cdot kg^{-1} \cdot d^{-1}$. All slopes were significant at $P = 0.05$.

Examination of individual data revealed a tendency for percent fecal water to increase with increased Zu/Preem intake.

Total Body Water and Body Fat

Individual total body water volumes (TBW) declined during Undernutrition and increased during Maintenance (Table 1.4). Four of five increased during Overnutrition (Table 1.4).

All calculated individual body fat percentages declined during Undernutrition and increased during Overnutrition (Table 1.4). The individual Maintenance changes in body fat were mixed, with no tendency for increase or decrease.

Water Transfer Rate

Water transfer rate and fractional body water turnover rate increased markedly with increased food intake (Table 1.1). A number of factors which could influence WTR were used as independent variables for linear regression and correlation analysis. These factors are listed in Table 1.5 in decreasing order of the strength of the linear relationship.

Electrolytes

Intake

Electrolyte concentrations in dry food were 4.4 ± 0.8 mg potassium · g^{-1} (n = 3) and 4.1 ± 0.6 mg sodium · g^{-1} (n = 3). The mean daily electrolyte intakes during Undernutrition were 35.6 mg · kg^{-1} potassium and 33.1 mg · kg^{-1} sodium. The mean daily intakes during Maintenance were 71.1 mg ·

Table 1.4. Total body water and body fat percent of captive wolves before and after fourteen days on each of three food intake levels[a] during winter.

Wolf	Parameter at Start of Undernutrition		Parameter at Start of Maintenance/End of Undernutrition	
	TBW[b] (l)	Fat[c] (%)	TBW (l)	Fat (%)
Rusty			20.72	20.7
Butch	23.45	19.9	22.16 ↓	19.3 ↓
Mix	20.93	16.3	19.63 ↓	17.0 ↓
Frosty	21.64	27.0	21.07 ↓	24.5 ↓
Mr. Brown	20.50	21.1	19.72 ↓	18.8 ↓
mean ± s.d.		21.6 ± 3.8		20.1 ± 2.8

Wolf	Parameter at Start of Overnutrition/End of Maintenance		Parameter at End of Overnutrition	
	TBW (l)	Fat (%)	TBW (l)	Fat (%)
Rusty	21.75 ↑	20.8 ↑	23.64 ↑	25.1 ↑
Butch	22.48 ↑	22.8 ↑	24.36 ↑	26.4 ↑
Mix	20.48 ↑	16.2 ↓	20.60 ↑	24.7 ↑
Frosty	23.70 ↑	19.4 ↓	21.99 ↓	29.3 ↑
Mr. Brown	20.00 ↑	22.4 ↑	21.05 ↑	29.0 ↑
mean ± s.d.		20.3 ± 2.0		26.9 ± 2.2

[a]Undernutrition food intake = 18.75 $g \cdot kg^{-1} \cdot d^{-1}$; Maintenance food intake = 37.50 $g \cdot kg^{-1} \cdot d^{-1}$; Overnutrition food intake = 75.00 $g \cdot kg^{-1} \cdot d^{-1}$. Each trial lasted 14 days. [b]TBW = total body water. [c]Fat = body fat as percent of body weight. Arrows (↑,↓) indicate direction of change from previous measurement.

TABLE 1.5. Linear regression and correlation of water transfer rate and factors which influence water transfer rate for captive wolves at three levels of food intake[a] during winter.

Independent Variable	Units	Slope	y-Intercept	Corr. Coef.	% of Var.[b]	Sample Size
Dry Matter Intake	$g \cdot kg^{-1} \cdot d^{-1}$	1.69	11.8	0.924	85.4	15
Total Potassium Loss[c]	$mg \cdot kg^{-1} \cdot d^{-1}$	0.46	2.1	0.914	83.5	55
Total Urine Potassium	$mg \cdot kg^{-1} \cdot d^{-1}$	0.46	5.9	0.860	74.0	58
Total Fecal Energy	$kcal \cdot kg^{-1} \cdot d^{-1}$	2.01	6.1	0.860	74.0	15
Total Urine Energy	$kcal \cdot kg^{-1} \cdot d^{-1}$	7.91	6.9	0.839	70.4	60
Total Sodium Loss[c]	$mg \cdot kg^{-1} \cdot d^{-1}$	0.47	10.7	0.836	69.9	55
Total Urine Nitrogen	$mg \cdot kg^{-1} \cdot d^{-1}$	0.040	7.4	0.831	69.1	60
Total Nitrogen Loss[c]	$mg \cdot kg^{-1} \cdot d^{-1}$	0.32	11.0	0.816	66.6	53
Total Urine Chloride	$mg \cdot kg^{-1} \cdot d^{-1}$	0.20	11.0	0.815	66.4	58
Total Urine Sodium	$mg \cdot kg^{-1} \cdot d^{-1}$	0.65	9.5	0.799	63.8	58
Total Fecal Nitrogen	$mg \cdot kg^{-1} \cdot d^{-1}$	0.16	27.0	0.697	48.6	55
Total Fecal Sodium	$mg \cdot kg^{-1} \cdot d^{-1}$	1.08	27.8	0.691	47.7	55
Total Fecal Potassium	$mg \cdot kg^{-1} \cdot d^{-1}$	2.48	24.8	0.668	44.6	55
Ambient Temperature	degrees C	1.6	74.7	0.549	30.1	190
Body Temperature	degrees C	14.2	- 497	0.327	10.7	1419

[a]Wolves spent 14 days on each of three food intake levels: 18.75 $g \cdot kg^{-1} \cdot d^{-1}$, 37.50 $g \cdot kg^{-1} \cdot d^{-1}$ and 75.00 $g \cdot kg^{-1} \cdot d^{-1}$. [b]% of Var. = percent of variability in water transfer rate which can be explained by variability in the independent variable. [c]Total Loss = sum of urine and fecal losses. All slopes were significant at P = 0.001.

kg^{-1} potassium and 66.3 mg • kg^{-1} sodium. During Overnutrition, the daily intakes were 142.2 mg • kg^{-1} potassium and 132.5 mg • kg^{-1} sodium. Chloride intake was not measured. The electrolyte intake from snow was assumed to be negligible.

Blood levels

Serum sodium, chloride, potassium and osmolality did not differ among the Nutrition trials (Tables 1.6 and 1.7).

Output

As food intake increased, mean total daily urine sodium, potassium and chloride losses increased (Tables 1.7 and 1.8). Urine electrolyte concentrations and osmolality did not differ among trials.

Mean total daily fecal sodium and potassium losses increased with increased food intake (Tables 1.7 and 1.9). Fecal electrolyte concentrations did not differ significantly among trials.

Nitrogen

Intake

The mean Zu/Preem nitrogen content was 6.20 ± 0.36% (n = 3) of the dry weight. The mean daily nitrogen intakes for Undernutrition, Maintenance and Overnutrition were therefore 501, 1002 and 2004 mg • kg^{-1}, respectively.

TABLE 1.6. Serum electrolyte concentrations and osmolalities of captive wolves at three levels of food intake[a] during winter.

Serum Parameter	Food Intake Trial		
	Undernutrition	Maintenance	Overnutrition
Sodium ($meq \cdot l^{-1}$)	147 ± 5 (20)	147 ± 2 (20)	148 ± 5 (20)
Potassium ($meq \cdot l^{-1}$)	4.5 ± 0.5 (20)	4.3 ± 0.4 (20)	4.1 ± 0.4 (20)
Chloride ($meq \cdot l^{-1}$)	115 ± 5 (20)	116 ± 10 (20)	117 ± 7 (20)
Osmolality ($mOsm \cdot kg^{-1}$)	307 ± 4 (20)	305 ± 3 (20)	305 ± 6 (20)

[a]Undernutrition food intake = 18.75 $g \cdot kg^{-1} \cdot d^{-1}$; Maintenance food intake = 37.50 $g \cdot kg^{-1} \cdot d^{-1}$; Overnutrition food intake = 75.00 $g \cdot kg^{-1} \cdot d^{-1}$. Each trial lasted 14 days. Data are given as mean ± standard deviation (sample size). Results of statistical comparisons among trials are in Table 1.7.

TABLE 1.7. Statistical comparison of electrolyte concentrations and losses, by analysis of variance, for captive wolves at three food intake levels[a] during winter.

	Food Intake Trials Compared		
Parameter	Undernutrition & Maintenance	Undernutrition & Overnutrition	Maintenance & Overnutrition
Serum Electrolyte Concentrations			
Sodium	NS	NS	NS
Potassium	NS	NS(4)	NS(4)
Chloride	NS	NS	NS
Serum Osmolality	NS	NS(3)	NS(3)
Urine Electrolyte Concentrations			
Sodium	NS	NS(4)	NS(4)
Potassium	NS(4)	NS(3)	NS
Chloride	NS(3)	NS(3)	NS(4)
Urine Osmolality	NS	NS(4)	NS(4)
Total Daily Urine Electrolyte Losses			
Sodium	*	*	*
Potassium	*(3)	*(4)	*(4)
Chloride	*	*	*
Fecal Electrolyte Concentrations			
Sodium	NS(4)	NS(3)	NS(4)
Potassium	NS	NS	NS
Total Daily Fecal Electrolyte Losses			
Sodium	*	*	*
Potassium	NS	*	*

[a]Undernutrition food intake = 18.75 $g \cdot kg^{-1} \cdot d^{-1}$; Maintenance food intake = 37.50 $g \cdot kg^{-1} \cdot d^{-1}$; Overnutrition food intake = 75.00 $g \cdot kg^{-1} \cdot d^{-1}$. Each trial lasted 14 days. Asterisk (*) indicates statistical significance at P = 0.05; NS indicates lack of statistical significance at P = 0.05. If statistical significance differed among the five wolves, results for the majority are shown, followed by number of wolves in parentheses. Data compared in this table are listed in Tables 1.6, 1.8, and 1.9.

TABLE 1.8. Urine electrolyte total losses and concentrations for captive wolves at three levels of food intake[a] during winter.

Parameter	Food Intake Trial		
	Undernutrition	Maintenance	Overnutrition
Total Urine Electrolytes Losses			
Sodium ($mg \cdot kg^{-1} \cdot d^{-1}$)	32.8 ± 4.6 (20)	47.8 ± 6.6 (20)	76.4 ± 24.3 (18)
Potassium ($mg \cdot kg^{-1} \cdot d^{-1}$)	49.9 ± 6.7 (20)	74.8 ± 9.3 (20)	120.9 ± 31.8 (18)
Chloride ($mg \cdot kg^{-1} \cdot d^{-1}$)	89.4 ± 15.5 (20)	148.8 ± 27.2 (20)	240.8 ± 67.0 (18)
Urine Electrolyte Concentrations			
Sodium ($meq \cdot l^{-1}$)	109 ± 28 (20)	113 ± 34 (20)	112 ± 30 (20)
Potassium ($meq \cdot l^{-1}$)	98 ± 25 (20)	103 ± 26 (20)	108 ± 28 (20)
Chloride ($meq \cdot l^{-1}$)	196 ± 60 (20)	225 ± 61 (20)	232 ± 63 (20)
Osmolality ($mOsm \cdot kg^{-1}$)	1927 ± 509 (20)	2057 ± 513 (20)	2002 ± 528 (20)

[a]Undernutrition food intake = 18.75 $g \cdot kg^{-1} \cdot d^{-1}$; Maintenance food intake = 37.50 $g \cdot kg^{-1} \cdot d^{-1}$; Overnutrition food intake = 75.00 $g \cdot kg^{-1} \cdot d^{-1}$. Each trial lasted 14 days. Data are given as mean ± standard deviation (sample size). Results of statistical comparisons are in Table 1.7.

TABLE 1.9. Fecal electrolyte total losses and concentrations for captive wolves at three levels of food intake[a] during winter.

Parameter	Food Intake Trial		
	Undernutrition	Maintenance	Overnutrition
Total Fecal Electrolytes Losses			
Sodium ($mg \cdot kg^{-1} \cdot d^{-1}$)	4.6 ± 2.3 (19)	13.9 ± 7.6 (20)	27.5 ± 10.8 (16)
Potassium ($mg \cdot kg^{-1} \cdot d^{-1}$)	3.7 ± 1.6 (19)	6.1 ± 3.0 (20)	12.8 ± 5.0 (16)
Fecal Electrolyte Concentrations			
Sodium (% dry fecal wt.)	0.27 ± 0.12 (19)	0.41 ± 0.21 (20)	0.41 ± 0.12 (20)
Potassium (% dry fecal wt.)	0.21 ± 0.03 (19)	0.17 ± 0.06 (20)	0.19 ± 0.05 (20)

[a]Undernutrition food intake = 18.75 $g \cdot kg^{-1} \cdot d^{-1}$; Maintenance food intake = 37.50 $g \cdot kg^{-1} \cdot d^{-1}$; Overnutrition food intake = 75.00 $g \cdot kg^{-1} \cdot d^{-1}$. Each trial lasted 14 days. Data are given as mean ± standard deviation (sample size). Results of statistical comparisons are in Table 1.7.

Output

Mean total urine nitrogen losses increased with increased food intake, while mean urine nitrogen concentrations did not differ among food intake levels (Tables 1.10 and 1.11). Mean fecal nitrogen concentration and total daily loss increased with increasing food intake (Tables 1.10 and 1.11).

Gross Energy

Intake

The gross energy content of dried Zu/Preem was 6.43 ± 0.06 kcal · g^{-1}. Daily gross energy intakes were 52 kcal · kg^{-1} for Undernutrition, 104 kcal · kg^{-1} for Maintenance and 208 kcal · kg^{-1} for Overnutrition.

Urine and Fecal Output

Mean total daily urine and fecal energy outputs increased with increasing food intake (Tables 1.11 and 1.12). Mean urine energy concentrations did not differ among trials, but mean fecal energy concentration increased with increased food intake.

Energy Expenditure

Mean daily energy expenditure increased with increased food intake but did not differ significantly among trials (Tables 1.11 and 1.12).

Electrolyte, Nitrogen and Energy Balances

Mean daily balances of sodium, potassium, nitrogen and energy increased with increasing food intake (Tables 1.13 and 1.14). The wolves

TABLE 1.10. Urine and fecal nitrogen total losses and concentrations for captive wolves at three levels of food intake[a] during winter.

Parameter	Food Intake Trial		
	Undernutrition	Maintenance	Overnutrition
Total Nitrogen Losses			
Urine ($mg \cdot kg^{-1} \cdot d^{-1}$)	529 ± 107 (20)	848 ± 87 (20)	1362 ± 283 (20)
Fecal ($mg \cdot kg^{-1} \cdot d^{-1}$)	35 ± 17 (19)	82 ± 37 (20)	198 ± 80 (16)
Nitrogen Concentrations			
Urine ($mg \cdot ml^{-1}$)	41.0 ± 10.6 (20)	43.2 ± 10.8 (20)	43.3 ± 11.1 (20)
Fecal (% dry fecal wt.)	2.0 ± 0.3 (19)	2.3 ± 0.5 (20)	3.0 ± 0.6 (20)

[a]Undernutrition food intake = 18.75 $g \cdot kg^{-1} \cdot d^{-1}$; Maintenance food intake = 37.50 $g \cdot kg^{-1} \cdot d^{-1}$; Overnutrition food intake = 75.00 $g \cdot kg^{-1} \cdot d^{-1}$. Each trial lasted 14 days. Data are given as mean ± standard deviation (sample size). Results of statistical comparisons are in Table 1.11.

TABLE 1.11. Statistical comparison of nitrogen parameters and energy parameters of captive wolves at three food intake levels[a] during winter.

Parameter	Food Intake Trials Compared: Undernutrition & Maintenance	Undernutrition & Overnutrition	Maintenance & Overnutrition
Nitrogen Concentrations			
Urine	NS	NS(4)	NS(4)
Feces	NS	*	*
Total Daily Nitrogen Losses			
Urine	*	*	*
Feces	NS(4)	*(4)	*
Energy Concentrations			
Urine	NS [b]	NS [b]	NS [b]
Feces	NS	*	*
Total Daily Energy Losses			
Urine	*	*	*
Feces	NS	*	*
Energy Expenditure	NS	NS	NS

[a]Undernutrition food intake = 18.75 $g \cdot kg^{-1} \cdot d^{-1}$; Maintenance food intake = 37.50 $g \cdot kg^{-1} \cdot d^{-1}$; Overnutrition food intake = 75.00 $g \cdot kg^{-1} \cdot d^{-1}$. Each trial lasted 14 days. Asterisk (*) indicates statistical significance at P = 0.05; NS indicates lack of statistical significance at P = 0.05. If statistical significance differed among the five wolves, results for the majority are shown, followed by number of wolves in parentheses. Data compared in this table are listed in Tables 1.9 and 1.11. [b]Significant first order interactions precluded ANOVA; examination of individual data and trial means revealed no apparent differences among trials.

TABLE 1.12. Urine and fecal energy total losses and concentrations plus energy expenditure of captive wolves at three levels of food intake[a] during winter.

Parameter	Food Intake Trial		
	Undernutrition	Maintenance	Overnutrition
Urine Total Loss ($kcal \cdot kg^{-1} \cdot d^{-1}$)	2.7 ± 0.5 (20)	4.3 ± 0.4 (20)	6.9 ± 1.4 (20)
Fecal Total Loss ($kcal \cdot kg^{-1} \cdot d^{-1}$)	11.7 ± 2.6 (5)	16.3 ± 2.4 (5)	28.0 ± 6.6 (5)
Urine Concentration ($kcal \cdot ml^{-1}$)	0.21 ± 0.05 (20)	0.22 ± 0.05 (20)	0.22 ± 0.06 (20)
Fecal Concentration ($kcal \cdot g^{-1}$)	4.4 ± 0.1 (5)	4.6 ± 0.1 (5)	4.9 ± 0.1 (5)
Energy Expenditure ($kcal \cdot kg^{-1} \cdot d^{-1}$)	59 ± 9 (4)	72 ± 28 (5)	102 ± 16 (5)

[a]Undernutrition food intake = 18.75 $g \cdot kg^{-1} \cdot d^{-1}$; Maintenance food intake = 37.50 $g \cdot kg^{-1} \cdot d^{-1}$; Overnutrition food intake = 75.00 $g \cdot kg^{-1} \cdot d^{-1}$. Each trial lasted 14 days. Data are given as mean ± standard deviation (sample size). Results of statistical comparisons are in Table 1.11.

TABLE 1.13. Electrolyte, nitrogen and energy balances of captive wolves at three levels of food intake[a] during winter.

Parameter	Food Intake Trial		
	Undernutrition	Maintenance	Overnutrition
Sodium Balance ($mg \cdot kg^{-1} \cdot d^{-1}$)	- 4.3 ± 5.3 (19)	4.4 ± 10.8 (20)	23.4 ± 25.8 (14)
Potassium Balance ($mg \cdot kg^{-1} \cdot d^{-1}$)	- 17.5 ± 6.5 (19)	- 12.7 ± 10.0 (20)	0.2 ± 21.6 (14)
Nitrogen Balance ($mg \cdot kg^{-1} \cdot d^{-1}$)	- 72 ± 104 (19)	105 ± 201 (20)	405 ± 326 (6)
Energy Balance ($kcal \cdot kg^{-1} \cdot d^{-1}$)	- 21.9 ± 6.4 (4)	11.7 ± 26.3 (5)	71.2 ± 16.3 (5)

[a]Undernutrition food intake = 18.75 $g \cdot kg^{-1} \cdot d^{-1}$; Maintenance food intake = 37.50 $g \cdot kg^{-1} \cdot d^{-1}$; Overnutrition food intake = 75.00 $g \cdot kg^{-1} \cdot d^{-1}$. Each trial lasted 14 days. Data are given as mean ± standard deviation (sample size). Results of statistical comparisons are in Table 1.14.

TABLE 1.14. Statistical comparison of balances and blood concentrations for captive wolves at three food intake levels[a] during winter.

Parameter	Food Intake Trials Compared		
	Undernutrition & Maintenance	Undernutrition & Overnutrition	Maintenance & Overnutrition
Balances			
Sodium	NS(3)	*(3)	*
Potassium	NS	*	*
Nitrogen	*	*	*
Energy	*	*	*
Blood Concentration			
Total Protein	NS(4)	NS(4)	NS
Hematocrit	*(3)	*(3)	NS

[a]Undernutrition food intake = 18.75 $g \cdot kg^{-1} \cdot d^{-1}$; Maintenance food intake = 37.50 $g \cdot kg^{-1} \cdot d^{-1}$; Overnutrition food intake = 75.00 $g \cdot kg^{-1} \cdot d^{-1}$. Each trial lasted 14 days. Asterisk (*) indicates statistical significance at P = 0.05; NS indicates lack of statistical significance at P = 0.05. If statistical significance differed among the five wolves, results for the majority are shown, followed by number of wolves in parentheses. If statistical significance differed among the four sample days, results for the majority are shown, followed by number of days in parentheses. Data compared in this table are listed in Tables 1.13 and 1.16.

were in positive sodium, nitrogen and energy balance when consuming the weight-maintaining diet (Maintenance) but were in negative potassium balance on the same diet (Table 1.15).

Hematocrit and Serum Total Protein Concentration

Hematocrit tended to decrease with increased food intake, but serum total protein concentration had no tendency to change (Tables 1.14 and 1.16). Mean hematocrit decreased significantly from Undernutrition to Maintenance and from Undernutrition to Overnutrition in three of five wolves. Only one wolf had significant differences in mean serum total protein concentration among the trials.

Body and Ambient Temperatures

Mean body temperatures for Undernutrition, Maintenance and Overnutrition were 37.8 ± 0.5C (n = 480), 37.9 ± 0.4C (n = 460) and 38.2 ± 0.3C (n = 479), respectively. Analysis of variance was not used because of significant first order interactions between trial and wolf and between trial and day. Four daily mean body temperatures from each trial were then examined for each wolf. Comparison of these means revealed a tendency for body temperature to increase with increasing food intake.

The mean ambient temperatures at approximately 0700 for Undernutrition, Maintenance and Overnutrition were -25.8 ± 5.9C (n = 12), -16.3 ± 3.8C (n = 13) and -15.5 ± 3.3C (n = 13), respectively.

TABLE 1.15. Calculated food intake associated with no net gain or loss (zero balance) in sodium, potassium, nitrogen and energy of captive wolves at three food intake levels[a] during winter, based on linear regression of balances with dry matter intake.

Dependent Variable	Units	Slope	Y-Intercept	Sample Size	Corr. Coef.	Zero Balance ($g \cdot kg^{-1} \cdot d^{-1}$) Dry	Wet
Sodium Balance	$mg \cdot kg^{-1} \cdot d^{-1}$	1.10	- 13.8	53	0.598	12.5	29.3
Potassium Balance	$mg \cdot kg^{-1} \cdot d^{-1}$	0.73	- 23.9	53	0.479	**32.7**[b]	**75.9**[b]
Nitrogen Balance	$mg \cdot kg^{-1} \cdot d^{-1}$	17.4	- 197	53	0.624	11.3	26.3
Energy Balance	$kcal \cdot kg^{-1} \cdot d^{-1}$	3.81	- 51.5	14	0.914	13.5	31.3

[a]The three food intake levels were 18.75 $g \cdot kg^{-1} \cdot d^{-1}$, 37.50 $g \cdot kg^{-1} \cdot d^{-1}$ and 75.00 $g \cdot kg^{-1} \cdot d^{-1}$, based on wet food intake. Each food intake trial lasted 14 days. Dry matter intake for linear regression was expressed in $g \cdot kg^{-1} \cdot d^{-1}$. [b]Zero balance food intakes exceeding the weight-maintaining diet (16.16 $g \cdot kg^{-1} \cdot d^{-1}$ dry, 37.50 $g \cdot kg^{-1} \cdot d^{-1}$ wet) are in **boldface**.

TABLE 1.16. Hematocrits and serum total protein concentrations of captive wolves at three levels of food intake[a] during winter.

Parameter	Food Intake Trial: Undernutrition	Maintenance	Overnutrition
Hematocrit (%)	53 ± 2 (20)	50 ± 1 (20)	50 ± 2 (20)
Total Protein ($g \cdot dl^{-1}$)	7.0 ± 0.3 (20)	7.0 ± 0.2 (20)	7.2 ± 0.3 (20)

[a] Undernutrition food intake = 18.75 $g \cdot kg^{-1} \cdot d^{-1}$; Maintenance food intake = 37.50 $g \cdot kg^{-1} \cdot d^{-1}$; Overnutrition food intake = 75.00 $g \cdot kg^{-1} \cdot d^{-1}$. Each trial lasted 14 days. Data are given as mean ± standard deviation (sample size). Results of statistical comparisons are in Table 1.14.

DISCUSSION

The Water Metabolism-Energetics Relationship

Water Transfer Rate and Dry Matter Intake

Dry matter intake was a driving force for water transfer rate in the wolves. Water transfer rate (Table 1.1) and five of the six water intakes and outputs (Tables 1.1 and 1.2) had strong linear relationships with dry matter intake (Table 1.3). A similar relationship has been reported in dogs: laboratory Beagles had a strong linear relationship between total water intake (excluding metabolic water) and total dry food intake over a food intake range from fasting to approximately twice maintenance (Cizek 1959). The Beagle study did not include low ambient temperatures or cold water consumption as variables.

Water transfer rate and the five water intakes and outputs had equally strong linear relationships with electrolyte, nitrogen and energy intakes. These linear relationships were strong because the electrolyte, nitrogen and energy intakes were functions of dry matter intake. A direct relationship between total water intake and energy intake has been demonstrated in laboratory dogs fed maintenance rations and rations 50% over maintenance (Adolph 1939, English and Filippich 1980). These canine studies differed from the wolf study in that the dogs were not subjected to undernutrition, cold ambient temperature or cold water consumption.

The strong linear relationships in the wolf study were attributed to absorption of osmotically active substances and to the osmotic action of urine and fecal constituents. Both processes would have increased plasma

osmolality and indirectly stimulated thirst by intracellular dehydration of the hypothalamic thirst center (Guyton 1976). Only one of the canine studies (Cizek 1959) addressed this mechanism: increasing the osmotic activity of the food with sodium chloride increased the total water intake (excluding metabolic water) in proportion to the amount of sodium chloride added.

More variation in WTR was attributed to variation in total daily losses of individual urine constituents than was attributed to total daily losses of any individual fecal constituent except fecal energy (Table 1.5). One explanation for more WTR variation being attributable to variation in urine constituent losses is that potassium could have been actively excreted into the gut without water (Fisher *et al.* 1976, Bastl *et al.* 1978). The high correlation of WTR with fecal energy could be attributed to osmotic drag exerted by unabsorbed and/or secreted organic molecules. These organic molecules would be determinants of fecal energy. None of the canine studies (Adolph 1939, Cizek 1959, English and Filippich 1980) examined the relationships between WTR and urine or fecal constituents.

Species Variation/Seasonal Adaptation in Water Transfer Rate

The wolves of this study had lower WTR in relation to dry matter intake than did dogs in the laboratory (Cizek 1959, English and Filippich 1980). Using the data of Cizek (1959), I estimated a linear equation of WTR and dry matter for comparison with the equation I derived for the wolves (Table 1.5). The y-intercepts were nearly identical, but the slope for dogs was at least 1.7 times the slope for wolves. The greater slope

for dogs indicates that the WTR-dry matter intake ratio in laboratory dogs was approximately twice that of the wolves.

The wolves also had lower total water intake in relation to energy intake than did laboratory dogs (Adolph 1939, English and Filippich 1980). In these canine studies, dry matter intake was measured as gross energy intake; for comparison, the wolf dry matter intakes were also expressed as gross energy intake. The total water intake-energy intake ratios of wolves were approximately half those of the dogs. The range of ratios for dogs was 0.73 to 1.15 ml $\cdot$ $kcal^{-1}$, compared to 0.48 $\pm$ 0.11 (n = 5), 0.38 $\pm$ 0.06 (n = 5) and 0.32 $\pm$ 0.05 (n = 5) for Undernutrition, Maintenance and Overnutrition, respectively.

The differences in WTR-dry matter intake ratios and total water intake-energy intake ratios between wolves and dogs were attributed to seasonal changes and/or species variation. Seasonal variation in WTR has been reported for humans (Yoshimura 1958), ruminants (Longhurst *et al.* 1970, Cameron and Luick 1972) and red-backed voles (Holleman *et al.* 1982a). Variation in WTR among related species within a given environment has also been documented (Macfarlane *et al.* 1971). It was not possible to differentiate between species and seasonal effects in dogs and wolves because species and season both differed. The dog-wolf comparisons evidence the need to separately study species and seasonal differences in the WTR-dry matter intake relationship.

Water Transfer Rate and Energy Expenditure

The mean WTR-energy expenditure ratios, in moles of water to moles of energy, were similar to those reported in the literature (Macfarlane *et al.* 1971, Kennedy and Macfarlane 1971). The mean wolf ratios were 2.2 for Undernutrition, 3.4 for Maintenance and 4.6 for Overnutrition (Table 1.17) compared to 3:1 for rodents and 5:1 for marsupials.

Water transfer rate and energy expenditure were not directly related in the wolves: linear regression and correlation analysis revealed no significant slope ($P = 0.05$). This finding contrasts the direct relationship between WTR and energy expenditure found among closely related species of mammals (excluding carnivores), birds and plants (Macfarlane *et al.* 1971, Kennedy and Macfarlane 1971). These direct relationships occurred among differing environments but have not been evaluated among differing food intake levels.

Losses of Body Water and Osmotically Active Substances

The total body water decrease during Undernutrition was not attributed to a decrease in vasopressin (antidiuretic hormone, ADH) activity. Vasopressin increases water uptake from the distal renal tubules and collecting ducts, so its suppression causes diuresis. If ADH-induced diuresis had occurred during Undernutrition, urine osmolality would have been lower than in the other two trials. Urine osmolality did not differ among trials.

The total body water decline during Undernutrition was therefore attributed to osmotic losses. Water was eliminated osmotically in urine

TABLE 1.17. Individual ratios of water transfer rate to energy expenditure of captive wolves at three food intake levels during winter.

Wolf	Food Intake Trial		
	Undernutrition	Maintenance	Overnutrition
Rusty		2.8	3.8
Butch	2.4	5.0	3.6
Mix	2.4	2.9	5.6
Frosty	2.1	2.1	5.1
Mr. Brown	3.2	6.5	7.4
mean ± s.d.	2.2 ± 0.5	3.4 ± 1.9	4.6 ± 1.7

Undernutrition food intake = 18.75 $g \cdot kg^{-1} \cdot d^{-1}$; Maintenance food intake = 37.50 $g \cdot kg^{-1} \cdot d^{-1}$; Overnutrition food intake = 75.00 $g \cdot kg^{-1} \cdot d^{-1}$. Each trial lasted 14 days. Ratios expressed in (moles water)·(moles energy)$^{-1}$.

and feces with sodium, potassium and nitrogen. All three osmotically active substances were in negative balance during Undernutrition. The urinary electrolyte and nitrogen losses during Undernutrition were 7.2 to 15.1 times greater than the fecal losses (Table 1.18), indicating that most of the net osmotic water loss was via the kidneys.

The mechanisms by which osmotically active substances were lost during Undernutrition were not clear. Nitrogen loss was attributed to catabolism of body protein. Potassium loss was attributed partially to cellular breakdown and partially to potassium loss from intact cells. Elkinton and Taffel (1942) documented potassium loss from intact cells during dehydration but were unable to explain the mechanism. The mechanism of sodium loss in the present study was unclear but was not attributed to decline in aldosterone activity. A decline in aldosterone activity would have decreased sodium reabsorption from the urine and increased urine sodium loss. Decreased sodium reabsorption would have decreased the urine potassium-sodium ratio because some of the reabsorbed sodium would have been exchanged for potassium. The lack of change in urine potassium-sodium ratio among the three trials (0.9 for all trials) indicated that there was no change in sodium reabsorption and therefore no change in aldosterone activity.

The difficulty in determining the mechanisms of electrolyte loss during undernutrition is not unique to this study. For example, natriuresis in early fasting (Tasker 1967, Cizek *et al.* 1977, Nohno *et al.* 1977) and in undernutrition (Michell 1981) has been reported, but its mechanism is still disputed. The possible mechanisms include cation loss obligatory to

TABLE 1.18. Ratio of urine to fecal total daily losses of sodium, potassium and nitrogen for captive wolves at three food intake levels during winter.

Food Intake Trial	Urine - Fecal Ratio		
	Sodium	Potassium	Nitrogen
Undernutrition	7.1	14	15.1
Maintenance	3.4	12	10.3
Overnutrition	2.8	9	6.9

Undernutrition food intake = 18.75 $g \cdot kg^{-1} \cdot d^{-1}$; Maintenance food intake = 37.50 $g \cdot kg^{-1} \cdot d^{-1}$; Overnutrition food intake = 75.00 $g \cdot kg^{-1} \cdot d^{-1}$. Each trial lasted 14 days.

catabolic anion loss (Sigler 1975), a natriuric effect of glucagon (Sigler 1975), temporary surplus from gut or bone (Chinn *et al.* 1970; Spark *et al.* 1975) and reduced sodium requirement in alimentary secretions (Michell 1981).

Tissue Hydration

Most wolves dehydrated while undereating and overeating, but the hydration changes during maintenance feeding were more variable (based on ΔTBW/ΔP, Table 1.19). Specific water compartments associated with these hydration changes could not be identified because no direct compartmental measurements were made.

Dehydration by the undernourished wolves was consistent with other studies. Pregnant rats on inadequate diets had net water losses (Lederman 1983). Conversely, water retention increased in undernourished humans (Keys *et al.* 1950) and ruminants (Farrell 1970, Cameron 1972, Cameron *et al.* 1975).

It is likely that dehydration during Undernutrition was due to hypothalamic thirst suppression. Thirst originates in the hypothalamus where there are many inputs regarding water and energy status. Hypothalamic inputs from the Undernutrition energy imbalance may have changed the hydration set-point and suppressed thirst. When energy balance improved during Maintenance, the changes in set point and thirst may have been partially or totally reversed.

The dehydration of Overnutrition was attributed to experimental design. It is likely that food quantity limited the wolf stomach capacity

TABLE 1.19. Changes in body weight, body water and hydration of captive wolves at three food intake levels during winter.

Trial	Wolf	ΔBW (kg)	ΔTBW (l)	ΔTBW/ΔLean ($l \cdot kg^{-1}$)	ΔTBW/ΔP ($l \cdot kg^{-1}$)
Undernutrition	Butch	- 2.3	- 1.29	0.820 ↓	12.9 ↓
Undernutrition	Mix	- 2.7	- 1.30	0.722 ↑	3.2 ↓
Undernutrition	Frosty	- 2.3	- 0.57	0.857 ↓	undef. ↓
Undernutrition	Mr. Brown	- 2.1	- 0.78	0.886 ↓	3.9 ↓
Maintenance	Rusty	+ 1.9	+ 1.03	0.607 ↓	3.4 ↑
Maintenance	Butch	+ 2.5	+ 0.33	0.500 ↓	0.5 ↓
Maintenance	Mix	+ 1.1	+ 0.85	0.680 ↓	4.2 ↑
Maintenance	Frosty	+ 1.8	+ 2.64	0.765 ↑	8.8 ↑
Maintenance	Mr. Brown	+ 2.3	+ 0.28	0.500 ↓	0.5 ↓
Overnutrition	Rusty	+ 5.2	+ 1.89		1.3 ↓
Overnutrition	Butch	+ 4.7	+ 1.87		2.7 NC
Overnutrition	Mix	+ 4.1	+ 0.12		0.1 ↓
Overnutrition	Frosty	+ 3.2	- 1.72		- 2.5 ↓
Overnutrition	Mr. Brown	+ 4.9	+ 1.05		0.5 ↓

Undernutrition food intake = 18.75 $g \cdot kg^{-1} \cdot d^{-1}$; Maintenance food intake = 37.50 $g \cdot kg^{-1} \cdot d^{-1}$; Overnutrition food intake = 75.00 $g \cdot kg^{-1} \cdot d^{-1}$. Each trial lasted 14 days. ΔBW = change in body weight; ΔTBW = change in total body water; ΔLean = change in lean body weight; ΔP = change in body dry protein. Arrows (↑,↓) indicate direction of change in body lean tissue water content; NC indicates no change.

for snow during the two hour daily access to food and snow. Had snow been offered for the same length of time but later in the day, the wolves may have consumed more snow and prevented dehydration.

Dehydration had no significant pathophysiological consequences in any trial. This statement was supported by three findings. The first finding was that dehydration was less than 1% of body weight in all wolves (Table 1.20). The first clinical signs of dehydration do not appear in dogs until 5% of body weight is lost (Kirk and Bistner 1975). The second finding was that serum sodium and potassium concentrations and serum osmolalities did not differ among the three trials or from the normal ranges for all wolves at the laboratory. Mansell and Clegg (1983) reported the third finding. Their cell culture dehydration studies indicated a lack of pathophysiological effects during dehydration: when cell volume was reduced by up to 35%, ultrastructure changed but viability and metabolism were little different from controls.

Snow Intake Suppression[1] and Energy Conservation During Undernutrition

There was no direct evidence of snow intake suppression during Undernutrition. The snow intake-dry matter intake ratio was not used because the ratio changed with food intake level. Failure of the straight

[1]See Suppressed Snow Intake in **Explanation of Terms**, page xix.

TABLE 1.20. Individual percent changes in body weight due to dehydration, calculated from ΔTBW/ΔLean for captive wolves at two levels of food intake during winter.

Food Intake Trial	Wolf	Percent Change in Body Weight
Undernutrition	Butch	- 0.5
Undernutrition	Mix	0.0
Undernutrition	Frosty	- 0.2
Undernutrition	Mr. Brown	- 0.4
Maintenance	Rusty	- 0.6
Maintenance	Butch	- 0.4
Maintenance	Mix	- 0.2
Maintenance	Frosty	+ 0.3
Maintenance	Mr. Brown	- 0.4

Undernutrition food intake = 18.75 $g \cdot kg^{-1} \cdot d^{-1}$; Maintenance food intake = 37.50 $g \cdot kg^{-1} \cdot d^{-1}$. Each trial lasted 14 days. ΔTBW = change in total body water (l); ΔLean = change in lean body weight (kg).

line of best fit to pass through the origin (Table 1.3) is evidence that the ratio changed with changing food intake.

There was, however, indirect evidence of suppressed snow intake during Undernutrition: dehydration, increased proportion of metabolic water production and decreased fecal water content. Dehydration was discussed in the previous section. The proportion of metabolic water was greater during Undernutrition than during Maintenance (Table 1.21); the difference in proportion was attributed to the greater proportion of fat catabolized during Undernutrition than during Maintenance (Table 1.22; Undernutrition catabolized fat:catabolized protein = 2.2 to >7.0, food fat:food protein = 1.1). The fecal water content of Undernutrition (58.1 ± 3.6%) was less than that of Maintenance (62.1 ± 5.3%).

The calculated quantity of energy saved by snow intake suppression was inconsequential. Even if a 40 kg wolf had become clinically dehydrated (5% of body weight), only 19 kcal · d^{-1} would have been saved [40 kg BW · 0.05 = 2.0 kg water lost; (132 kcal · kg $water^{-1}$) · (2.0 kg water · 14 d^{-1}) = 19 kcal · d^{-1}]. None of the wolves was clinically dehydrated during Undernutrition, so less than 19 kcal · d^{-1} for a 40 kg wolf was saved. Despite the greater metabolic water proportion, the snow fraction during Undernutrition was only one percentage point less than during Maintenance, and the means were not significantly different (ANOVA). Even if the difference had been significant, only about 1 kcal · d^{-1} would have been saved in a 40 kg wolf. The lower fecal water content of Undernutrition, compared to that of Maintenance, would have saved less than 1 kcal · d^{-1}. The total energy saved by snow intake suppression was

TABLE 1.21. Water intakes as percent of water transfer rate for captive wolves at three food intake levels[a] during winter.

Compartment	Food Intake Trial		
	Undernutrition	Maintenance	Overnutrition
Snow Water	28.7 ± 21.5 (69)	30.2 ± 19.6 (70)	33.0 ± 18.0 (69)
Food Water	45.8 ± 7.5 (69)	53.6 ± 7.1 (70)	58.0 ± 11.4 (69)
Metabolic Water	25.4 ± 18.2 (69)	16.2 ± 16.9 (70)	9.1 ± 18.0 (69)

[a] Undernutrition food intake = 18.75 $g \cdot kg^{-1} \cdot d^{-1}$; Maintenance food intake = 37.50 $g \cdot kg^{-1} \cdot d^{-1}$; Overnutrition food intake = 75.00 $g \cdot kg^{-1} \cdot d^{-1}$. Each trial lasted 14 days. Data are given as mean ± standard deviation (sample size).

TABLE 1.22. Individual changes in body composition of captive wolves during each of three trials during winter where food intake level[a] differed.

Wolf	Food Intake Trial								
	Undernutrition			Maintenance			Overnutrition		
	Body Composition Changes[b]			Body Composition Changes			Body Composition Changes		
	ΔF (kg)	ΔP (kg)	ΔO (kg)	ΔF (kg)	ΔP (kg)	ΔO (kg)	ΔF (kg)	ΔP (kg)	ΔO (kg)
Rusty				+ 0.4	+ 0.3	+ 0.2	+ 2.9	+1.5	- 1.1
Butch	- 0.7	- 0.1	- 0.2	+ 1.9	+ 0.7	- 0.4	+ 2.7	+ 0.7	- 0.6
Mix	- 0.9	- 0.4	- 0.1	- 0.1	+ 0.2	+ 0.2	+ 3.8	+ 0.9	- 0.7
Frosty	- 1.6	0.0	- 0.1	- 1.6	+ 0.3	+ 0.5	+ 4.9	+ 0.7	- 0.7
Mr. Brown	- 1.2	- 0.2	+ 0.1	+ 1.7	+ 0.2	+ 0.1	+ 3.8	+ 2.0	- 1.9

[a] Undernutrition food intake = 18.75 $g \cdot kg^{-1} \cdot d^{-1}$; Maintenance food intake = 37.50 $g \cdot kg^{-1} \cdot d^{-1}$; Overnutrition food intake = 75.00 $g \cdot kg^{-1} \cdot d^{-1}$. Each trial lasted 14 days. [b] ΔF = change in body fat, ΔP = change in body protein, ΔO = change in body nonfat nonprotein solids.

less than 0.5 kcal · kg^{-1} · d^{-1}. This quantity was an inconsequential fraction (less than 1%) of the estimated daily energy expenditure during Undernutrition (59 ± 9 kcal · kg^{-1} · d^{-1}, Table 1.12).

It is likely that energy conservation and thus snow intake suppression by the wolves were influenced by the relative timing of snow and food consumption. Since food and snow were offered together for two hours every day, some or all of the energy used to melt ingested snow and raise its temperature could have resulted from the calorigenic effect of the food. As a result, metabolic heat would have been conserved, and snow intake suppression should have been lower than if snow had been offered after most of the heat due to calorigenic effect had been dissipated.

Heat loss during digestion has not been shown to balance the energy cost of snow consumption in canids. Studies of sheep (Degen and Young 1981), however, suggested that at least some of the energy cost of consuming snow was balanced by heat lost during digestion.

Result of Testing the Hypothesis

Energy expenditure was reduced and snow intake was suppressed during Undernutrition. Energy expenditure was reduced by less than 1%. The estimated maximal snow intake suppression in Undernutrition was 0.4 ml · kg^{-1} · d^{-1} by dehydration, 0.3 ml · kg^{-1} · d^{-1} by change in water intake proportions and 0.2 ml · kg^{-1} · d^{-1} by decrease in fecal water content. The total, 0.9 ml · kg^{-1} · d^{-1}, was 11% of the mean snow intake during Undernutrition. These results supported the hypothesis that wolves reduce energy expenditure by suppressing snow intake when energy balance is

negative during winter.

CONCLUSIONS

1. Water transfer rate and five of six water intakes and outputs had strong linear relationships with dry matter intake in captive wolves during winter. Food intake varied from undernutrition to overnutrition. The strong linear relationships were attributed primarily to absorption and elimination of osmotically active food components.

2. Water transfer rate and daily energy expenditure of the wolves were not linearly related.

3. When undernourished in winter, captive wolves suppressed snow intake through dehydration, decreased fecal water content and perhaps decreased proportion of snow intake. Less than 1% of the daily energy budget was conserved by snow intake suppression during undernutrition. The estimated maximal snow intake suppression during undernutrition was approximately 11% of the mean daily snow intake. These results support the hypothesis that wolves reduce energy expenditure by suppressing snow intake when energy balance is negative during winter.

Chapter 2

Water Metabolism of Captive Wolves in Winter.

II. Effects of Gorging and Fasting.

INTRODUCTION

The previous chapter addressed water metabolism of captive wolves when daily food intake varied between undernutrition and overnutrition. Water transfer rate and snow intake were directly related to dry matter intake, but there was indirect evidence that snow intake and dry matter intake were uncoupled during undernutrition: wolves dehydrated, fecal water content increased and snow intake proportion may have declined slightly, compared to the daily maintenance diet. The degree of uncoupling was not great enough to preclude the direct relationships. Uncoupling implied that snow intake was suppressed during negative energy balance (undernutrition).

Fasting and alternate gorging-fasting may also influence water metabolism of captive wolves. Both conditions should decrease energy balance relative to daily eating and are therefore expected to suppress snow intake. Alternate gorging-fasting is expected to be less efficient than daily eating because conversion of food first to body stores and then to energy should yield less net energy than conversion of food directly to energy. This comparison has not been made for canids, but alternate gorging-fasting yielded less net energy than did daily feeding of the same total ration in sheep (Watson *et al.* 1975). Alternate gorging-fasting could therefore cause negative energy balance while daily feeding of the same average daily quantity could maintain energy balance at zero or slightly positive. Since negative energy balance caused by undernutrition

suppressed snow intake, it is likely that negative energy balance caused by fasting or alternate gorging-fasting will also suppress snow intake.

Fasting and alternate gorging-fasting are considered in this experiment because wild wolves often gorge and fast. Prey availability and hunting success do not always permit wild wolves to feed daily (Mech 1970). Stomach capacity and digestion rate permit an adult wolf to consume 9 kilograms of meat in an hour (Mech 1970) and 20 kilograms in 24 hours (Zimen 1981). These quantities are far in excess of the estimated minimum daily amount required for an adult wolf in winter, 1.4 to 1.9 kg (Mech 1970). After consuming a carcass, wolves may fast for more than five days before making another kill (Mech 1970, Zimen 1981).

The effects of fasting on water metabolism in canids are better understood than the effects of alternate gorging-fasting. Daily voluntary water intake of fasted laboratory dogs ranged from 20% to 35% of water intake (fresh water plus water in food) of dogs fed maintenance diets (Kleitman 1927, Cizek 1959). This information is of limited value in predicting how snow intake of wolves would be affected by fasting in winter because the canine studies included neither low ambient temperatures nor cold water. There is little information on how fasting affects body water content in canids. Body water content changes were either not considered (Kleitman 1927, Cizek 1959) or were inconclusive (Prentiss *et al.* 1959). The effect of alternate gorging-fasting on water metabolism in wolves is even more difficult to predict because there have been no such studies in canids.

Information on noncanid species is also of limited value in predicting the effects of fasting or alternate gorging-fasting on water metabolism of wolves in winter. The effects of fasting on body water percent were inconsistent among species. In cats, total body water percent did not change after three weeks of food deprivation and ad libitum water (Prentiss et al. 1959). Under similar circumstances, total body water percent increased in white rats and mice (Prentiss et al. 1959) and decreased in lactating goats (Chaiyabutr et al. 1980). Species differences preclude extrapolation of gorging-fasting effects on water transfer rate from noncanids to canids. Sheep fed a seven-day ration once a week had higher total water intake and urine production over seven days than did sheep fed the same total amount of food in seven equal daily portions (Watson et al. 1975). It is not known whether wolves respond similarly during winter or have evolved physiological mechanisms which prevent increased total water intake with alternate gorging-fasting.

This experiment was designed to provide some of the missing information on how water metabolism is affected by fasting and alternate gorging-fasting in canids. It tested the hypothesis that wolves reduce energy expenditure by suppressing snow intake when in negative energy balance during winter. There were two objectives: to determine the effects on winter water metabolism when captive wolves fasted and to determine the effects on winter water metabolism when wolves alternately gorged and fasted.

METHODS

Experimental Animals

The five wolves used were those described in Chapter 1. In the present experiment, the animals were ten years old. Housing and care were as described in Chapter 1.

Experimental Conditions

There were three trials: one control trial and two gorge-fast trials. During the control trial (late November), the wolves were offered a maintenance Zu/Preem ration (37.5 mg · kg^{-1}, Chapter 1) two hours daily for seven days. The food was always warmed to approximately body temperature before feeding, to simulate the temperature of freshly killed prey. During the two gorge-fast trials, the wolves were alternately gorged and fasted, the length of the gorge-fast cycle differing between trials. In the 4-day gorge-fast trial (early to mid January), a four day maintenance ration was fed in approximately 500 g portions as often as the wolves would consume the food with little or no vomiting. Thus, food was offered approximately every six hours until all was consumed. This gorging period required approximately 36 hours. The wolves were then fasted until four full days after the gorging had begun. This gorge-fast cycle was repeated three times. The trial began and ended on the last day of a fast, having a total length of 17 days. The 8-day gorge-fast trial (mid to late March) was similar to the 4-day gorge-fast trial except that an eight day ration was fed in approximately four days, and the wolves fasted

for the remainder of the eight days. The cycle was repeated once. The 8-day gorge-fast trial began and ended on the last day of a fast, so the total duration was again 17 days.

All three trials were conducted in an unheated building and were preceded by conditioning periods. The control conditioning period lasted seven days. The wolves were kept in metabolism cages in the ambient temperature building and fed a maintenance ration daily. Prior to each gorge-fast trial, the wolves were conditioned to alternate gorging and fasting for eight days outside then for twelve (4-day gorge-fast trial) days or eight (8-day gorge-fast) days in the indoor metabolism cages.

Snow was offered for two hours at about 1400 every day of every trial. The snow container was weighed before and after to determine amount consumed. As in the Chapter 1 trials, the experimental day was begun when the snow was removed.

Body Weight

Each wolf was weighed on alternate mornings during the control trial. The 4-day gorge-fast body weights were recorded every day for two consecutive gorge-fast cycles and on the last day of five consecutive cycles. (Weight recordings were obtained on the last day of five cycles because the trial began on the last day of a cycle and continued for four complete cycles.) The 8-day gorge-fast body weights were recorded on alternate days for one cycle and on the last day of three consecutive cycles. (Weight recordings were obtained on the last day of three cycles because the trial began on the last day of a cycle and continued for two

complete cycles.) For weighing, each wolf was moved into an aluminum transport cage and placed on a platform balance. Two metal trays covered the bottom of the transport cage for collection of urine and feces.

Body Temperature

The equipment and methods used to measure body temperature were described in Chapter 1. During the control trial, body temperatures were recorded hourly for one 24 hour period. During the 4-day gorge-fast trial, hourly measurements were taken for four consecutive days. During the 8-day gorge-fast trial, body temperature was recorded every six hours for the entire trial.

Ambient Temperature

Ambient temperature was measured as described in Chapter 1 every time a body temperature was recorded.

Urine, Fecal, Blood and Food Analyses

Sample Collection

Blood was sampled as described in Chapter 1 on alternate days in the control trial. During the gorge-fast trials, samples were collected every day of the first gorge-fast cycle and on the final day of each fasting period.

Urine was collected daily as described in Chapter 1 and allowed to warm to approximately room temperature. Specific gravity was measured

with a refractometer. Feces were collected daily as described in Chapter 1 except that there were no 24 hour collections.

Samples for chemical analyses of urine and feces were collected on the same days as blood samples. The Chapter 1 results of food chemical analyses and food water content measurements were used for the present experiment.

Hematocrit

Hematocrit was measured as described in Chapter 1.

Chemical Analyses

The blood parameters measured were serum electrolyte concentration (sodium, potassium, chloride), serum osmolality and serum total protein concentration. Urine parameters measured were electrolyte concentrations (sodium, potassium, chloride), osmolality and nitrogen concentration. Fecal parameters measured were concentrations of sodium, potassium and nitrogen. The analytical methods used were described in Chapter 1.

Water Intake and Output

Water intake measurements were similar to those in Chapter 1. The only difference was that calculations of oxidative water were not made.

Water losses were determined as in Chapter 1. Urine water content was calculated from the linear equation of Chapter 1 urine water content (UW, %) and urine specific gravity (SG):

$$UW = -152.8\ SG + 251.7\ (r = 0.577,\ P < 0.001,\ n = 208) \qquad (2.1).$$

Total Body Water, Body Fat and Water Transfer Rate

Total body water (TBW) was determined through the use of tritiated water (TOH) for the first and last days of each trial. Each injection was intravenous (cephalic venapuncture) and consisted of approximately 500 µCi TOH mixed with sodium radiosulfate ($Na_2{}^{35}SO_4$) in 2.0 ml. The injection syringe was rinsed with normal saline which was also injected. Three or four blood samples were taken from the opposite cephalic vein at variable intervals 1.5 to 4.0 hours postinjection and processed as described in Chapter 1. The mean specific activity for each wolf was used in Equation 1.1 to calculate TBW. Blood samples for determining daily fractional body water turnover rate (k, d^{-1}) and WTR were collected on alternate control and 8-day gorge-fast trial days and daily in the 4-day gorge-fast trial. Fractional turnover rate and WTR were calculated using Equations 1.2 and 1.3, as described in Chapter 1.

Body fat was calculated according to Equation 1.4.

Extracellular Fluid Space

Functional extracellular fluid space (ECF) was estimated as the radiosulfate space for the first and last days of each trial. Each wolf was injected intravenously with approximately 200 µCi radiosulfate, and four blood samples were taken at variable intervals 1.5 to 5.0 hours post-injection. The blood was centrifuged, the plasma was removed and plasma proteins were precipitated with 20% trichloroacetic acid. One milliliter of supernatant was mixed with 10.0 ml scintillation cocktail (Aquasol II,

New England Nuclear) and 0.25 ml glacial acetic acid in a scintillation vial. The mixture was placed in a liquid scintillation counter and simultaneously counted for TOH and radiosulfate. Disintegrations per minute at zero time (t_0) were calculated by extrapolation.

The specific activity of radiosulfate at t_0 in each sample was used to calculate functional ECF space according to Equation 2.2:

$$ECF = S_I \cdot F_1 \cdot F_2 \cdot V \cdot S_S^{-1} \cdot F_3^{-1} \quad (2.2),$$

where

ECF = functional ECF space in ml

S_I = specific activity of injection stock solution, in DPM $\cdot$ ml^{-1}

$$= \frac{\text{(DPM diluted stock solution)} \cdot \text{(dilution factor)}}{\text{(volume stock sample counted)}}$$

S_S = specific activity of plasma sample, in DPM $\cdot$ ml^{-1},

$$= \frac{\text{(sample DPM)}}{\text{(sample volume, 0.5 ml)}}$$

F_1 = Donnan factor of sulfate = 0.90 (Ryan *et al.* 1956, Bauer *et al.* 1975a)

F_2 = correction factor for water content of protein = 0.93 (Ryan *et al.* 1956, Bauer *et al.* 1975a)

F_3 = correction factor for DPM of sample (F_3 corrects for change in liquid volume after plasma protein precipitation with 20% TCA.) = 0.97

V = volume of stock solution injected (ml).

Simultaneous Counting of Tritium and Radiosulfate

Standard dual label counting methods were used to simultaneously count tritium and sulfur-35. For tritium, the lower energy isotope, the upper window was set to maximize efficiency while minimizing sulfur-35 detection. The sulfur-35 channel was set for maximum sulfur-35 detection and insignificant tritium detection. The DPM values were calculated as follows (Tracor Analytic):

$$DPM_1 = (N_A \cdot EFF_{2B} - N_B \cdot EFF_{2A}) \cdot EFF_{1A}^{-1} \cdot EFF_{2B}^{-1} \quad (2.3)$$

$$DPM_2 = N_B \cdot EFF_{2B}^{-1} \quad (2.4),$$

where

DPM_1 = calculated DPM for tritium

DPM_2 = calculated DPM for radiosulfate

N_A = net CPM in tritium channel

N_B = net CPM in radiosulfate channel

EFF_{1A} = fractional counting efficiency for tritium in the tritium channel

EFF_{2A} = fractional counting efficiency for tritium in the radiosulfate channel

EFF_{2B} = fractional counting efficiency for radiosulfate in the radiosulfate channel.

(Note: There is no EFF_{1B} because the number of tritium counts in the radiosulfate channel was insignificant.)

Efficiencies (EFF) were calculated from the external standard ratios (ESR) automatically determined for each channel by the scintillation counter. The external standard was ^{133}Ba. Quench curves (ESR versus EFF)

were constructed for each isotope with the use of quenched standards. These standards were counted with each set of samples so that changes in the quench curves would be detected.

Plasma Volume

Plasma volume was estimated for the first and last days of each trial with the use of radioiodinated (^{125}I) human serum albumin (RIHSA) (Ames Company, Elkhart, Indiana). The RIHSA was supplied in syringes containing 2.5 µCi in 1.5 ml.

Approximately 0.1 ml of RIHSA was removed from each syringe for determination of percent ^{125}I uptake to albumin. Each uptake sample was diluted to 1.0 ml with saline and counted in a gamma scintillation counter (Model 1197, Tracor Analytic). Protein was then precipitated from the solution with 20% TCA, and the sample was centrifuged. The supernatant was removed and counted in the gamma counter. Percent uptake was calculated with Equation 2.5:

$$\% \text{ uptake} = \frac{(\text{original DPM}) - (\text{supernatant DPM})}{(\text{original DPM})} \cdot 100\% \qquad (2.5).$$

Prior to RIHSA injection, syringes containing the labelled albumin were placed in a gamma counter for determination of injected activity. These counts were corrected for resolving time loss and for geometry. Resolving time loss was calculated with Equation 2.6 (Wang *et al.* 1975):

$$n = m \cdot (1-mT)^{-1} \qquad (2.6),$$

where

n = corrected counting rate (sec^{-1})

m = observed counting rate (sec^{-1})

T = resolving time (taken as $7.5 \cdot 10^{-6}$ sec).

Geometry corrections were made in a Model 1195 gamma counter (Tracor Analytic). A ^{125}I standard point source was counted at six positions in a gamma counting tube. These positions were at the levels of the 0.00, 0.35, 0.70, 1.05 and 1.40 ml marks on the original RIHSA-containing syringes and at the center of a 1.0 ml volume in a gamma counting tube. The resulting correction factor placed the counts of 1.4 ml RIHSA (the injected volume) in terms of 1.0 ml (the volume of plasma counted) in a glass counting vial.

RIHSA injection into a cephalic vein by venapuncture was performed as described for TOH and radiosulfate. One RIHSA injection syringe was used for each wolf. After RIHSA injection into the wolves, the empty syringes, plastic catheters and needles were counted for uninjected activity. Blood was collected from the opposite cephalic vein at 15, 20 and 25 minutes post-injection. The blood was immediately placed in a heparinized test tube, then centrifuged and the plasma withdrawn.

One milliliter plasma samples were counted in the Model 1197 gamma counter. Counting time was set to give at least 10,000 accumulated counts, and all samples were counted twice. A ^{125}I standard was counted with every sample group and with every group of RIHSA injection syringes to determine counting efficiency. Observed activities (CPM) were divided by the decimal efficiency to give DPM.

Plasma volume was calculated with Equation 2.7:

$$PV = A_I \cdot V_1 \cdot F_1 \cdot F_2 \cdot A_S^{-1} + V_2 \qquad (2.7),$$

where

PV = plasma volume (ml)

A_I = total activity injected (DPM)

A_S = activity of plasma sample (DPM)

V_1 = volume of plasma sample (ml)

V_2 = average net volume lost due to blood samples = 17 ml

F_1 = correction factor for free ^{125}I in the RIHSA syringe, calculated as 0.01 · uptake (%)

F_2 = correction factor for ^{125}I decay from date of preinjection syringe count to date of sample count. The value for A_S was taken as the average of the 15, 20 and 25 minute samples.

Calculations

Calculations of daily electrolyte and nitrogen losses and balances were described in Chapter 1.

Most calculations of change in body protein (ΔP), body fat (ΔF) and body nonprotein nonfat solids (ΔO) were performed as described in Chapter 1. The exceptions were calculations of ΔP for the two gorge-fast trials, which were performed with Equations 2.8 and 2.9:

ΔP (4-day gorge-fast trial) = [sum of (net daily Nitrogen balance · BW) for 4 days of one cycle] · 4 cycles · 0.00000625 kg protein · mg N^{-1} (2.8)

ΔP (8-day gorge-fast trial) = [sum of (net bidaily Nitrogen balance · mean bidaily BW) · 2] · 2 cycles · 0.00000625 kg protein · mg N^{-1} (2.9).

The ratios ΔTBW/ΔLean and ΔTBW/ΔP were used to evaluate the overall tissue hydration changes in each trial, as described in Chapter 1.

Calculation of control energy expenditure was as described in Chapter 1. Calculation of overall gorge-fast energy expenditure was similar. The only difference was that total rather than daily energy expenditure was calculated for each gorge-fast trial. This total was divided by the number of days in the trial.

Statistical Analyses

Data were compared by analysis of variance at P = 0.05. Where three or more groups were compared and found significantly different, the means were compared in pairs using the Least Significant Difference at P = 0.05. Where there was significant first order interaction between wolf and treatment, the data were partitioned by wolf, and ANOVA was repeated. Tests for overall changes during each gorge-fast trial were made with data from the last fasting day of each cycle in the trial.

RESULTS

Most results are presented as differences among gorging, fasting and the control trial and as differences in overall changes among the three trials. Overall changes are those occurring from beginning to end of a trial. Electrolyte and nitrogen outputs are presented as differences in concentrations and in total daily losses among gorging, fasting and the control trial; outputs are also presented as differences in overall total daily losses among the three trials.

Body Weight

The only significantly different mean body weights were between gorging and fasting. Gorging body weights were significantly greater than fasting body weights in both gorge-fast trials (Figures 2.1 and 2.2).

Water Intakes and Outputs

The two most obvious findings regarding mean daily snow intake were the increase in gorging over fasting and the spike on gorging day two (Tables 2.1 and 2.2). These findings were in both gorge-fast trials.

The changes in mean daily water intake in food were similar to the changes in mean daily snow intake (Table 2.1). Mean daily water consumption in food was highest in gorging, intermediate in the control trial and lowest in fasting. There was no spike on gorging day two as there was for snow intake.

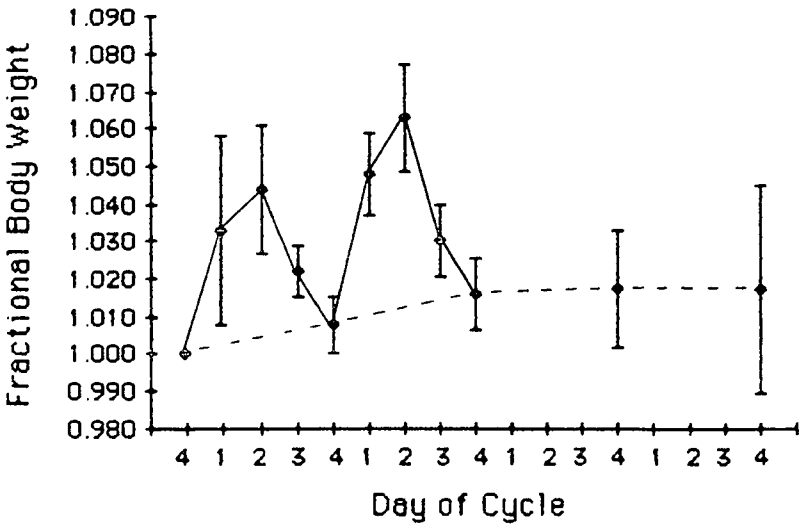

Figure 2.1. Fractional body weights of captive wolves when gorging and fasting in a 4-day gorge-fast cycle. Weights are fractions of weight on day 1 of trial. Error bars indicate standard deviation. Solid lines connect daily measurements; dashed lines connect body weights on each final fasting day.

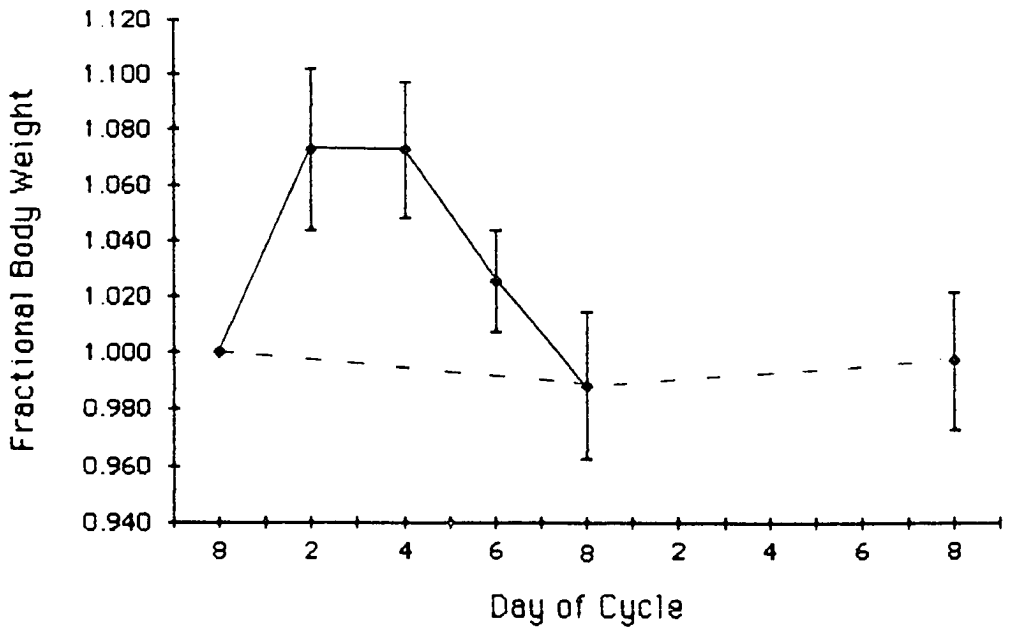

Figure 2.2. Fractional body weights of captive wolves when gorging and fasting in an 8-day gorge-fast cycle. Weights are fractions of weight on day 1 of trial. Error bars indicate standard deviation. Solid lines connect bidaily measurements; dashed lines connect body weights on each final fasting day.

TABLE 2.1. Water intakes of captive wolves alternately gorged and fasted or fed daily[a] during winter.

Feeding Trial	Water Intake ($ml \cdot kg^{-1} \cdot d^{-1}$)		
	Snow Water	Food Water	Metabolic Water
Control	18.1 ± 9.8 (35)	20.7 ± 0.8 (35)	3.8 ± 7.4 (35)
4-Day Gorge-Fast			
Gorging	30.9 ± 18.8 (10)	42.3 ± 1.1 (10)	- 10.8 ± 30.3 (10)
Fasting	13.0 ± 14.5 (10)	0	10.0 ± 14.7 (10)
8-Day Gorge-Fast			
Gorging	25.6 ± 19.4 (20)	39.0 ± 22.7 (20)	8.3 ± 28.3 (20)
Fasting	4.0 ± 3.8 (18)	0	3.8 ± 3.9 (18)
4-Day Gorge-Fast			
Day 1	15.7 ± 13.3 (5)	42.9 ± 0.5 (5)	14.2 ± 19.0 (5)
Day 2	46.2 ± 6.5 (5)	41.6 ± 1.2 (5)	- 35.9 ± 11.5 (5)
Day 3	17.8 ± 18.4 (5)	0	20.7 ± 10.8 (5)
Day 4	8.1 ± 8.5 (5)	0	- 0.7 ± 9.1 (5)
8-Day Gorge-Fast			
Day 1	18.4 ± 6.4 (5)	60.5 ± 11.1 (5)	3.0 ± 18.5 (5)
Day 2	45.4 ± 19.8 (5)	54.9 ± 9.8 (5)	- 18.4 ± 13.0 (5)
Day 3	29.4 ± 16.1 (5)	31.2 ± 11.4 (5)	3.3 ± 16.8 (5)
Day 4	9.1 ± 13.7 (5)	9.5 ± 7.0 (5)	45.3 ± 18.3 (5)
Day 5	4.2 ± 2.9 (4)	0	2.0 ± 5.8 (4)
Day 6	2.2 ± 2.6 (4)	0	4.1 ± 6.0 (4)
Day 7	3.7 ± 3.6 (5)	0	5.4 ± 2.1 (5)
Day 8	5.7 ± 5.4 (5)	0	3.5 ± 1.4 (5)

[a] During the Control trial, wolves were fed a weight-maintaining diet daily; during the 4-Day Gorge-Fast trial, wolves were fed twice the maintenance diet for two days (Gorging) and fasted for two days (Fasting); during the 8-Day Gorge-Fast trial, wolves were fed twice the maintenance diet for four days (Gorging) and fasted for four days (Fasting). Data are given as mean ± standard deviation (sample size). Results of statistical comparisons are in Table 2.2.

TABLE 2.2. Statistical comparisons of body water intake and output parameters, by analysis of variance among trials, for captive wolves alternately gorged and fasted or fed daily[a] during the winter.

	Trials Compared							
Parameter	C v. 4D G	C v. 4D F	C v. 8D G	C v. 8D F	4D G v. 8D G	4D F v. 8D F	4D G v. 4D F	8D G v. 8D F
Water Intake								
Snow Water	*	NS	NS	*	NS	NS(3)	*	*
Food Water	*	*	*	*	NS	NS	*	*
Metabolic Water	*	NS	NS	NS	NS	NS	NS	NS
Water Output								
Urine Water	NS(3)	NS(3)	*	*	NS	NS	NS(3)	*
Fecal Water	*(4)	*	*	*	NS	NS	*	*
Evaporative Water	NS	NS	*	*	NS	*	NS	*
Fecal Water Content	*(4)	NS(4)	*	*	NS	NS	NS(4)	*
Water Transfer Rate	NS	NS	*	*	NS	NS	*	*
Fractional Turnover Rate	*	NS	*	*	NS	NS	*	*

[a]During the Control (C), wolves were fed a weight-maintaining diet daily; during the 4-Day Gorge-Fast trial (4D), wolves were fed twice the maintenance diet for two days (4D G) and fasted for two days (4D F); during the 8-Day Gorge-Fast trial (8D), wolves were fed twice the maintenance diet for four days (8D G) and fasted for four days (8D F). Asterisk (*) indicates statistical significance at P = 0.05; NS indicates lack of statistical significance at P = 0.05. If statistical significance differed among the five wolves, results for majority are shown, followed by number of wolves in parentheses. Data compared in this table are listed in Tables 2.1, 2.3, 2.4 and 2.7.

The most noticeable aspects of calculated mean daily metabolic water production were marked negative values on the second gorging day and unusually high values on the day food intake ceased (Table 2.1). These two events occurred in both gorge-fast trials.

Mean daily water losses in urine, feces and evaporation were all highest during gorging, intermediate during the control trial and lowest during fasting (Table 2.3). All differences were statistically significant for the control trial versus the 8-day gorge-fast trial (Table 2.2). Only the fecal water difference was statistically significant for the control trial versus the 4-day gorge-fast trial.

Mean fecal water content was highest during fasting, intermediate during gorging and lowest during the control trial (Table 2.4). These differences among gorging, fasting and control were significant for the 8-day gorge-fast data but not for the 4-day gorge-fast data (Table 2.2).

Total Body Water and Body Fat

All individual TBWs declined during the control trial. During the gorge-fast trials, most individual TBWs declined to a lesser extent than in the control trial and a few increased (Tables 2.5 and 2.6).

All individual body fat percentages increased during the control trial and four of five increased during both gorge-fast trials (Table 2.5).

TABLE 2.3. Water outputs of captive wolves alternately gorged and fasted or fed daily[a] during the winter.

Feeding Trial	Water Output ($ml \cdot kg^{-1} \cdot d^{-1}$)		
	Urine Water	Fecal Water	Evaporative Water
Control	20.4 ± 8.7 (35)	6.5 ± 2.4 (35)	15.7 ± 5.3 (35)
4-Day Gorge-Fast			
Gorging	30.3 ± 17.7 (10)	14.4 ± 4.4 (10)	17.6 ± 21.5 (10)
Fasting	12.6 ± 5.7 (9)	2.4 ± 1.8 (9)	9.6 ± 21.8 (9)
8-Day Gorge-Fast			
Gorging	31.1 ± 18.6 (20)	13.4 ± 6.0 (20)	28.4 ± 24.0 (20)
Fasting	9.1 ± 5.4 (18)	1.2 ± 1.4 (18)	- 2.4 ± 8.3 (18)
4-Day Gorge-Fast			
Day 1	27.1 ± 15.2 (5)	13.8 ± 5.1 (5)	32.0 ± 13.4 (5)
Day 2	33.6 ± 21.0 (5)	15.0 ± 4.1 (5)	3.2 ± 18.5 (5)
Day 3	15.1 ± 5.2 (5)	3.0 ± 1.7 (5)	20.4 ± 24.3 (5)
Day 4	9.6 ± 5.3 (4)	1.7 ± 1.7 (4)	- 3.9 ± 6.3 (4)
8-Day Gorge-Fast			
Day 1	18.3 ± 6.7 (5)	8.1 ± 2.7 (5)	55.5 ± 2.7 (5)
Day 2	36.5 ± 22.2 (5)	18.9 ± 7.1 (5)	26.5 ± 18.0 (5)
Day 3	41.0 ± 26.1 (5)	12.4 ± 3.7 (5)	10.6 ± 24.8 (5)
Day 4	28.7 ± 7.9 (5)	14.3 ± 4.8 (5)	20.9 ± 6.7 (5)
Day 5	14.4 ± 6.5 (4)	1.9 ± 2.0 (4)	- 10.5 ± 11.2 (4)
Day 6	9.2 ± 3.7 (4)	2.5 ± 0.8 (4)	- 5.5 ± 5.2 (4)
Day 7	5.5 ± 2.6 (5)	0.2 ± 0.5 (5)	3.4 ± 4.6 (5)
Day 8	8.2 ± 5.7 (5)	0.7 ± 0.6 (5)	0.3 ± 6.2 (5)

[a] During the Control trial, wolves were fed a weight-maintaining diet daily; during the 4-Day Gorge-Fast trial, wolves were fed twice the maintenance diet for two days (Gorging) and fasted for two days (Fasting); during the 8-Day Gorge-Fast trial, wolves were fed twice the maintenance diet for four days (Gorging) and fasted for four days (Fasting). Data are given as mean ± standard deviation (sample size). Results of statistical comparisons are in Table 2.2.

TABLE 2.4. Fecal water contents for captive wolves alternately gorged and fasted or fed daily[a] during winter.

Food Intake	Fecal Water Content (%)
Control	63.7 ± 2.0 (35)
4-Day Gorge-Fast	
Gorging	66.2 ± 2.6 (40)
Fasting	73.7 ± 11.5 (38)
8-Day Gorge-Fast	
Gorging	66.6 ± 2.9 (40)
Fasting	76.6 ± 10.5 (28)

[a] During the Control trial, wolves were fed a weight-maintaining diet daily; during the 4-Day Gorge-Fast trial, wolves were fed twice the maintenance diet for two days (Gorging) and fasted for two days (Fasting); during the 8-Day Gorge-Fast trial, wolves were fed twice the maintenance diet for four days (Gorging) and fasted for four days (Fasting). Data are given as mean ± standard deviation (sample size). Results of statistical comparisons are in Table 2.2.

TABLE 2.5. Total body water and body fat of captive wolves alternately gorged and fasted or fed daily[a] during winter.

Trial	Wolf	Initial Trial Values TBW[b] (l)	Initial Trial Values Fat[c] (%)	Final Trial Values TBW[b] (l)	Final Trial Values Fat[c] (%)
C	Rusty	23.02	25.1	22.78 ↓	28.3 ↑
C	Butch	23.70	23.8	22.75 ↓	28.0 ↑
C	Mix	21.56	19.1	21.33 ↓	20.8 ↑
C	Frosty	22.37	29.6	21.97 ↓	34.2 ↑
C	Mr. Brown	22.42	24.9	21.29 ↓	28.5 ↑
	mean ± st. dev.	-	24.5 ± 3.7	-	28.0 ± 4.8
4-D	Rusty	20.74	32.2	20.93 ↑	34.3 ↑
4-D	Butch	22.74	26.7	22.30 ↓	30.6 ↑
4-D	Mix	19.38	22.4	19.33 ↓	23.2 ↑
4-D	Frosty	21.04	36.1	20.80 ↓	35.4 ↓
4-D	Mr. Brown	20.52	31.1	20.55 ↑	31.7 ↑
	mean ± st. dev.	-	29.7 ± 5.3	-	31.0 ± 4.8
8-D	Rusty	21.01	29.6	21.14 ↑	30.2 ↑
8-D	Butch	22.46	22.1	22.32 ↓	23.8 ↑
8-D	Mix	19.31	16.8	19.25 ↓	17.8 ↑
8-D	Frosty	21.62	31.3	20.87 ↓	31.0 ↓
8-D	Mr. Brown	20.99	28.9	20.66 ↓	29.4 ↑
	mean ± st. dev.	-	27.5 ± 6.1	-	26.4 ± 5.6

[a] During the Control trial (C), wolves were fed a weight-maintaining diet daily; during the 4-Day Gorge-Fast trial (4-D), wolves were fed a four-day maintenance ration in two days and fasted for two days; during the 8-Day Gorge-Fast trial (8-D), wolves were fed an eight-day ration in four days and fasted for four days. [b]TBW = total body water. [c]Fat = body fat (% of body weight). Arrows (↑, ↓) indicate direction of change from initial to final measurement within each trial. For each trial, initial and final mean Fat were not significantly different (analysis of variance, $P = 0.05$).

TABLE 2.6. Changes in total body water and body water subcompartments of captive wolves alternately gorged and fasted or fed daily[a] during winter.

Trial	Wolf	ΔTBW[b] (l)	ΔTBW[c] ($l \cdot d^{-1}$)	ΔECF[d] (l)	ΔICF[e] (l)	ΔPV[f] (l)	ΔISF[g] (l)
C	Rusty	- 0.24	- 0.04	- 1.06	+ 0.82	+ 1.25	- 2.31
C	Butch	- 0.95	- 0.16	- 0.73	- 0.22	+ 1.27	- 2.00
C	Mix	- 0.23	- 0.04	- 0.26	+ 0.03	+ 1.68	- 1.94
C	Frosty	- 0.40	- 0.07	- 0.05	- 0.35	+ 2.04	- 2.09
C	Mr. Brown	- 1.13	- 0.19	- 0.66	- 0.47	+ 0.78	- 1.44
4-D	Rusty	+ 0.19	+ 0.01	+ 0.17	+ 0.02	+ 1.11	- 0.94
4-D	Butch	- 0.23	- 0.01	+ 0.27	- 0.50	+ 0.20	+ 0.07
4-D	Mix	- 0.05	- < 0.01	+ 0.16	- 0.21	+ 0.99	- 0.83
4-D	Frosty	- 0.25	- 0.02	+ 0.36	- 0.61	- 0.05	+ 0.41
4-D	Mr. Brown	+ 0.02	+ < 0.01	- 0.58	+ 0.60	+ 1.03	- 1.61
8-D	Rusty	+ 0.13	+ 0.01	- 0.29	+ 0.42	- 0.73	+ 0.44
8-D	Butch	- 0.14	- 0.01	- 0.40	+ 0.26	- 1.65	+ 1.25
8-D	Mix	- 0.06	- < 0.01	- 0.26	+ 0.20	-	-
8-D	Frosty	- 0.75	- 0.05	+ 0.46	- 1.21	+ 0.06	+ 0.40
8-D	Mr. Brown	- 0.32	- 0.02	- 0.87	+ 0.55	+ 1.84	- 2.71

[a] During the Control trial (C), wolves were fed a weight-maintaining diet daily; during the 4-Day Gorge-Fast trial (4-D), wolves were fed a four-day maintenance ration in two days and fasted for two days; during the 8-Day Gorge-Fast trial (C), wolves were fed an eight-day ration in four days and fasted for four days. [b]ΔTBW = change in total body water; [c]ΔTBW ($l \cdot d^{-1}$) represents mean daily change in TBW; [d]ΔECF = change in extracellular fluid volume; [e]ΔICF = change in intracellular fluid volume; [f]ΔPV = change in plasma volume; [g]ΔISF = change in interstitial fluid volume. Changes are from beginning to end of trial. .

Water Transfer Rate and Daily Fractional Turnover Rate

Mean WTR and fractional turnover rate were highest during gorging, intermediate during the control trial and lowest during fasting (Tables 2.2 and 2.7).

Body Water Subcompartments

Functional Extracellular Fluid Space

All individual ECFs declined during the control trial, but the overall ECF changes during the gorge-fast trials were mixed (Table 2.8). Most individual ECFs increased during the 4-day gorge-fast trial and declined during the 8-day gorge-fast trial.

Plasma Volume

All individual PVs increased during the control trial, but the overall changes during the gorge-fast trials were mixed (Table 2.9). Most individual PVs increased during the 4-day gorge-fast trial. There were equal numbers of increases and decreases during the 8-day gorge-fast trial.

Intracellular Fluid Volume

Overall individual ICF changes were mixed in all three trials. Most individual ICFs decreased during the control trial and 4-day gorge-fast trial and increased during the 8-day gorge-fast trial (Table 2.10).

TABLE 2.7. Water transfer rate and daily fractional body water turnover rate for captive wolves alternately gorged and fasted or fed daily[a] during winter.

Feeding Trial	Water Transfer Rate ($ml \cdot kg^{-1} \cdot d^{-1}$)	Fractional Turnover Rate (d^{-1})
Control	42.6 ± 8.5 (5)	0.077 ± 0.013 (5)
4-Day Gorge-Fast		
Gorging	62.4 ± 20.4 (10)	0.121 ± 0.037 (10)
Fasting	22.9 ± 24.6 (10)	0.044 ± 0.046 (10)
8-Day Gorge-Fast		
Gorging	72.9 ± 21.2 (10)	0.134 ± 0.035 (10)
Fasting	7.9 ± 4.5 (9)	0.014 ± 0.008 (9)

[a] During the Control trial , wolves were fed a weight-maintaining diet daily; during the 4-Day Gorge-Fast trial, wolves were fed twice the maintenance diet for two days (Gorging) and fasted for two days (Fasting); during the 8-Day Gorge-Fast trial, wolves were fed twice the maintenance diet for four days (Gorging) and fasted for four days (Fasting). Data are given as mean ± standard deviation (sample size). Results of statistical comparisons are in Table 2.2.

TABLE 2.8. Functional extracellular fluid volumes of captive wolves alternately gorged and fasted or fed daily[a] during winter.

Trial	Wolf	ECF (l) Beginning of Trial	ECF (l) End of Trial
Control	Rusty	9.12	8.07 ↓
Control	Butch	8.02	7.29 ↓
Control	Mix	8.12	7.85 ↓
Control	Frosty	7.83	7.79 ↓
Control	Mr. Brown	8.51	7.85 ↓
4-Day Gorge-Fast	Rusty	7.39	7.57 ↑
4-Day Gorge-Fast	Butch	8.13	8.37 ↑
4-Day Gorge-Fast	Mix	7.60	7.77 ↑
4-Day Gorge-Fast	Frosty	7.61	7.97 ↑
4-Day Gorge-Fast	Mr. Brown	8.38	7.80 ↓
8-Day Gorge-Fast	Rusty	7.72	7.44 ↓
8-Day Gorge-Fast	Butch	7.80	7.40 ↓
8-Day Gorge-Fast	Mix	7.36	7.10 ↓
8-Day Gorge-Fast	Frosty	8.10	8.56 ↑
8-Day Gorge-Fast	Mr. Brown	8.15	7.28 ↓

[a] During the Control, wolves were fed a weight-maintaining diet daily; during the 4-Day Gorge-Fast trial, wolves were fed a four-day maintenance ration in two days and fasted two days; during the 8-Day Gorge-Fast trial, wolves were fed an eight-day ration in four days and fasted for four days. Arrows (↑, ↓) indicate direction of change from beginning to end of a trial.

TABLE 2.9. Plasma volumes of captive wolves alternately gorged and fasted or fed daily[a] during winter.

Trial	Wolf	PV (l) Beginning of Trial	PV (l) End of Trial
Control	Rusty	2.09	3.34 ↑
Control	Butch	2.12	3.39 ↑
Control	Mix	1.96	3.64 ↑
Control	Frosty	1.89	3.93 ↑
Control	Mr. Brown	2.07	2.85 ↑
4-Day Gorge-Fast	Rusty	3.26	4.39 ↑
4-Day Gorge-Fast	Butch	3.01	3.21 ↑
4-Day Gorge-Fast	Mix	3.09	4.09 ↑
4-Day Gorge-Fast	Frosty	2.88	2.82 ↓
4-Day Gorge-Fast	Mr. Brown	3.96	4.99 ↑
8-Day Gorge-Fast	Rusty	5.22	4.48 ↓
8-Day Gorge-Fast	Butch	4.66	3.01 ↓
8-Day Gorge-Fast	Mix	4.51	-
8-Day Gorge-Fast	Frosty	3.89	3.95 ↑
8-Day Gorge-Fast	Mr. Brown	4.13	5.97 ↑

[a] During the Control, wolves were fed a weight-maintaining diet daily; during the 4-Day Gorge-Fast trial, wolves were fed a four-day maintenance ration in two days and fasted two days; during the 8-Day Gorge-Fast trial, wolves were fed an eight-day ration in four days and fasted for four days. Arrows (↑, ↓) indicate direction of change from beginning to end of a trial.

TABLE 2.10. Intracellular fluid volumes of captive wolves alternately gorged and fasted or fed daily[a] during winter.

Trial	Wolf	ICF (l) Beginning of Trial	ICF (l) End of Trial
Control	Rusty	13.90	14.71 ↑
Control	Butch	15.68	15.46 ↓
Control	Mix	13.44	13.48 ↑
Control	Frosty	14.54	14.18 ↓
Control	Mr. Brown	13.91	13.44 ↓
4-Day Gorge-Fast	Rusty	13.35	13.36 ↑
4-Day Gorge-Fast	Butch	14.41	13.93 ↓
4-Day Gorge-Fast	Mix	11.78	11.56 ↓
4-Day Gorge-Fast	Frosty	13.43	12.83 ↓
4-Day Gorge-Fast	Mr. Brown	12.14	12.75 ↑
8-Day Gorge-Fast	Rusty	13.29	13.70 ↑
8-Day Gorge-Fast	Butch	14.66	14.92 ↑
8-Day Gorge-Fast	Mix	11.95	12.15 ↑
8-Day Gorge-Fast	Frosty	13.52	12.31 ↓
8-Day Gorge-Fast	Mr. Brown	12.84	13.38 ↑

[a] During the Control, wolves were fed a weight-maintaining diet daily; during the 4-Day Gorge-Fast trial, wolves were fed a four-day maintenance ration in two days and fasted two days; during the 8-Day Gorge-Fast trial, wolves were fed an eight-day ration in four days and fasted for four days. Arrows (↑, ↓) indicate direction of change from beginning to end of a trial.

Interstitial Fluid Volume

All individual ISFs declined during the control trial, and the overall changes during the gorge-fast trials were mixed (Table 2.11). Most individual ISFs declined during the 4-day gorge-fast trial and increased during the 8-day gorge-fast trial.

Electrolytes

Intake

Electrolyte concentrations in the food dry matter were 4.3 ± 0.8 mg potassium · g^{-1} (n = 3) and 4.1 ± 0.7 mg sodium · g^{-1} (n = 3). Daily potassium intakes (mg · kg^{-1}) were 68.1 for the control trial and 139.2 during gorging. Daily sodium intakes (mg · kg^{-1}) were 66.2 for the control trial and 134.1 during gorging. The electrolyte intake from snow was assumed to be negligible. Chloride intake was not measured.

Blood Levels

The most notable serum electrolyte differences between gorging and fasting were in sodium concentration and in osmolality (Tables 2.12 and 2.13). The 4-day gorge-fast gorging mean sodium concentration was significantly greater than the fasting mean. The 8-day gorge-fast sodium concentrations had the same relationship, but they were not significantly different. The 4- and 8-day gorge-fast mean gorging osmolalities were significantly greater than the corresponding mean fasting osmolalities and the mean control osmolality.

TABLE 2.11. Interstitial fluid volumes of captive wolves alternately gorged and fasted or fed daily[a] during winter.

Trial	Wolf	ISF (l) Beginning of Trial	ISF (l) End of Trial
Control	Rusty	7.03	4.73 ↓
Control	Butch	5.90	3.90 ↓
Control	Mix	6.16	4.21 ↓
Control	Frosty	5.94	3.86 ↓
Control	Mr. Brown	6.44	5.00 ↓
4-Day Gorge-Fast	Rusty	4.11	3.18 ↓
4-Day Gorge-Fast	Butch	5.12	5.16 ↑
4-Day Gorge-Fast	Mix	4.51	3.68 ↓
4-Day Gorge-Fast	Frosty	4.73	5.15 ↑
4-Day Gorge-Fast	Mr. Brown	4.42	2.81 ↓
8-Day Gorge-Fast	Rusty	2.50	2.96 ↑
8-Day Gorge-Fast	Butch	3.14	4.39 ↑
8-Day Gorge-Fast	Mix	2.85	-
8-Day Gorge-Fast	Frosty	4.21	4.61 ↑
8-Day Gorge-Fast	Mr. Brown	4.02	1.31 ↓

[a] During the Control, wolves were fed a weight-maintaining diet daily; during the 4-Day Gorge-Fast trial, wolves were fed a four-day maintenance ration in two days and fasted two days; during the 8-Day Gorge-Fast trial, wolves were fed an eight-day ration in four days and fasted for four days. Arrows (↑, ↓) indicate direction of change from beginning to end of a trial.

TABLE 2.12. Serum electrolyte concentrations and serum osmolalities for captive wolves alternately gorged and fasted or fed daily[a] during winter.

Feeding Trial	Sodium Concentration ($meq \cdot l^{-1}$)	Potassium Concentration ($meq \cdot l^{-1}$)	Chloride Concentration ($meq \cdot l^{-1}$)	Osmolality ($mOsm \cdot kg^{-1}$)
Control	149 ± 3 (20)	4.3 ± 0.3 (20)	121 ± 7 (20)	296 ± 16 (20)
4-Day Gorge-Fast				
Gorging	150 ± 6 (20)	4.0 ± 0.5 (20)	126 ± 7 (20)	324 ± 21 (20)
Fasting	141 ± 8 (20)	3.9 ± 0.4 (20)	122 ± 10 (20)	300 ± 6 (20)
8-Day Gorge-Fast				
Gorging	152 ± 5 (10)	4.3 ± 0.4 (10)	126 ± 7 (10)	316 ± 12 (10)
Fasting	149 ± 3 (10)	4.6 ± 0.4 (10)	129 ± 12 (10)	303 ± 4 (10)

[a] During the Control, wolves were fed a weight-maintaining ration daily; during the 4-Day Gorge-Fast trial, wolves were fed a twice maintenance ration for two days (Gorging) and fasted for two days (Fasting); during the 8-Day Gorge-Fast trial, wolves were fed a twice maintenance ration for four days (Gorging) and fasted for four days (Fasting). Data are given as mean ± standard deviation (sample size). Results of statistical comparisons are in Table 2.13.

TABLE 2.13. Statistical comparisons (analysis of variance) of electrolyte concentrations and losses for captive wolves alternately gorged and fasted or fed daily[a] during winter.

	Trials Compared							
Parameter	C v. 4D G	C v. 4D F	C v. 8D G	C v. 8D F	4D G v. 8D G	4D F v. 8D F	4D G v. 4D F	8D G v. 8D F
Serum Electrolyte Concentrations								
Sodium	NS	*	NS	NS	NS	*	*	NS
Potassium	*	*	NS	*	NS	*	NS	NS
Chloride	*	NS	NS	NS(4)	NS	NS	NS	NS
Serum Osmolality	*	NS(3)	*(3)	NS	NS	NS	*	*
Urine Electrolyte Concentrations								
Sodium	NS	*	NS	*	NS	NS	NS	*
Potassium	NS	*	NS	*	NS	NS(4)	*	*
Chloride	NS	NS	NS	*(3)	*	*	NS(4)	*(4)
Urine Osmolality	NS	*(3)	NS	*	NS	NS(3)	NS	*
Total Daily Urine Losses								
Sodium	NS	*	*	*	NS	*	*	*
Potassium	*	*	*	*	*	NS	*	*
Chloride	NS	*	*	*	*	*	*	*
Fecal Electrolyte Concentrations								
Sodium	NS	*	NS	NS(3)	NS	NS	*	NS(3)
Potassium	NS(3)	*	NS	NS(3)	*	NS	*	*
Total Daily Fecal Electrolyte Losses								
Sodium	*	*	NS(3)	*	NS	NS	*	*
Potassium	*	NS	*(3)	*	NS(4)	NS	*(3)	*

[a] During the Control (C), wolves were fed a weight-maintaining diet daily; during the 4-Day Gorge-Fast trial (4D), wolves were fed twice the maintenance diet for two days (4D G) and fasted for two days (4D F); during the 8-Day Gorge-Fast trial (8D), wolves were fed twice the maintenance diet for four days (8D G) and fasted for four days (8D F). Asterisk (*) indicates statistical significance, and NS indicates lack of statistical significance (P = 0.05). If statistical significance differed among the five wolves, results for majority are shown, followed by number of wolves in parentheses. Data compared in this table are listed in Tables 2.12, 2.14 and 2.15.

There were significant overall increases in mean serum sodium concentration and mean serum osmolality during the 8-day gorge-fast trial.

Urine Output

The most significant findings regarding urine electrolyte concentrations were the low mean sodium, potassium and chloride concentrations and low mean osmolality during fasting (Tables 2.13 and 2.14). These mean concentrations were lower during fasting than during gorging or during the control trial. The statistical relationships show that these differences were more pronounced for sodium, potassium and osmolality than for chloride (Table 2.13). The differences were also more pronounced for the 4-day gorge-fast trial than for the 8-day gorge-fast trial (Table 2.13). There were no overall changes in mean electrolyte concentrations or mean osmolality in either gorge-fast trial.

Mean total daily urine sodium, potassium and chloride losses were highest during gorging, intermediate during the control trial and lowest during fasting (Table 2.14). The only differences not statistically significant were total sodium and total chloride for the control trial versus 4-day gorge-fast gorging (Table 2.13).

There was a significant overall increase in mean total daily urine sodium loss during the 8-day gorge-fast trial. There were no significant overall changes in mean total daily urine sodium losses during the 4-day gorge-fast trial. There were no significant overall changes for potassium or chloride losses in either gorge-fast trial.

TABLE 2.14. Urine electrolyte concentrations, osmolalities and total daily electrolyte losses for captive wolves alternately gorged and fasted or fed daily[a] during winter.

Feeding Trial	Concentrations			
	Sodium ($meq \cdot l^{-1}$)	Potassium ($meq \cdot l^{-1}$)	Chloride ($meq \cdot l^{-1}$)	Osmolality ($mOsm \cdot kg^{-1}$)
Control	110 ± 39 (20)	96 ± 26 (20)	175 ± 53 (20)	1733 ± 512 (20)
4-Day Gorge-Fast				
Gorging	106 ± 59 (20)	93 ± 39 (20)	145 ± 65 (20)	1727 ± 603 (20)
Fasting	76 ± 44 (20)	69 ± 29 (20)	141 ± 63 (20)	1395 ± 476 (20)
8-Day Gorge-Fast				
Gorging	104 ± 36 (20)	99 ± 30 (20)	225 ± 85 (10)	1777 ± 526 (20)
Fasting	54 ± 28 (20)	72 ± 32 (20)	95 ± 31 (15)	1250 ± 406 (20)

	Total Losses		
	Sodium ($mg \cdot kg^{-1} \cdot d^{-1}$)	Potassium ($mg \cdot kg^{-1} \cdot d^{-1}$)	Chloride ($mg \cdot kg^{-1} \cdot d^{-1}$)
Control	59 ± 16 (18)	78 ± 17 (18)	133 ± 32 (18)
4-Day Gorge-Fast			
Gorging	66 ± 29 (20)	103 ± 37 (20)	135 ± 70 (20)
Fasting	22 ± 17 (20)	33 ± 19 (20)	62 ± 37 (19)
8-Day Gorge-Fast			
Gorging	84 ± 33 (20)	134 ± 48 (20)	270 ± 127 (10)
Fasting	13 ± 8 (20)	24 ± 8 (20)	31 ± 21 (10)

[a] During the Control, wolves were fed a weight-maintaining ration daily; during the 4-Day Gorge-Fast trial, wolves were fed a twice maintenance ration for two days (Gorging) and fasted for two days (Fasting); during the 8-Day Gorge-Fast trial, wolves were fed a twice maintenance ration for four days (Gorging) and fasted for four days (Fasting). Data are given as mean ± standard deviation (sample size). Results of statistical comparisons are in Table 2.13.

Fecal Output

Mean fecal sodium and potassium concentrations were highest during fasting and did not differ between gorging and the control trials (Table 2.15). Differences between fasting means and gorging or control means were more prevalent during the 4-day gorge-fast trial than during the 8-day gorge-fast trial (Table 2.13).

Mean total daily fecal sodium and potassium losses were greatest during gorging, intermediate during the control trial and least during fasting (Tables 2.13 and 2.15).

Since there were insufficient data from the gorge-fast trials, no analyses were done for overall changes in fecal electrolyte concentrations or total daily losses.

Nitrogen

Intake

Nitrogen concentration in the food dry matter was 62.0 ± 3.6 mg · g^{-1} (n = 3). The daily nitrogen intakes were 1003 mg · kg^{-1} during the control trial and 2006 mg · kg^{-1} during gorging. Nitrogen intake from snow was assumed to be zero.

Output

The most notable findings regarding urine and fecal nitrogen concentrations occurred during fasting, where mean urinary nitrogen

TABLE 2.15. Fecal electrolyte concentrations and total daily losses for captive wolves alternately gorged and fasted or fed daily[a] during winter.

	Concentrations (% Dry Fecal Wt.)		Total Losses ($mg \cdot kg^{-1} \cdot d^{-1}$)	
Feeding Trial	Sodium	Potassium	Sodium	Potassium
Control	0.36 ± 0.10 (15)	0.13 ± 0.05 (15)	10.9 ± 4.9 (15)	4.0 ± 1.7 (15)
4-Day Gorge-Fast				
Gorging	0.40 ± 0.13 (10)	0.24 ± 0.04 (10)	26.6 ± 11.5 (10)	16.1 ± 5.3 (10)
Fasting	1.16 ± 1.09 (10)	0.41 ± 0.22 (10)	5.9 ± 4.8 (10)	2.6 ± 2.0 (10)
8-Day Gorge-Fast				
Gorging	0.56 ± 0.44 (10)	0.16 ± 0.10 (10)	40.1 ± 22.3 (10)	13.0 ± 9.5 (10)
Fasting	1.54 ± 1.38 (8)	0.37 ± 0.22 (8)	4.6 ± 3.2 (8)	1.2 ± 0.5 (8)

[a] During the Control, wolves were fed a weight-maintaining ration daily; during the 4-Day Gorge-Fast trial, wolves were fed double rations for two days (Gorging) and fasted for two days (Fasting); during the 8-Day Gorge-Fast trial, wolves were fed double rations for four days (Gorging) and fasted for four days (Fasting). Data are given as mean ± standard deviation (sample size). Results of statistical comparisons are listed in Table 2.13.

concentrations were lowest and mean fecal nitrogen concentrations highest (Tables 2.16 and 2.17). The control means did not differ from the gorging means.

Mean total daily nitrogen losses in both urine and feces were greatest during gorging, intermediate during the control trial and least during fasting (Tables 2.16 and 2.17).

There was no evidence of overall change in mean urine nitrogen concentration or mean total daily urine nitrogen losses from beginning to end of either gorge-fast trial. There were not enough data to test for overall changes in fecal nitrogen.

Energy Expenditure

Mean overall gorging-fasting energy expenditures were similar for the 4- and 8-day gorge-fast trials: 67 ± 20 (n = 5) and 75 ± 7 (n = 5) kcal · kg^{-1} · d^{-1}, respectively. These mean overall expenditures were more than three times the control energy expenditure of 20 ± 24 (n = 4) kcal · kg^{-1} · d^{-1}.

Electrolyte and Nitrogen Balances

All balances were highest during gorging, intermediate in the control trial and lowest during fasting (Tables 2.17 and 2.18). The differences in balance among food intake levels were greatest for nitrogen.

TABLE 2.16. Nitrogen in urine and feces of captive wolves alternately gorged and fasted or fed daily[a] during winter.

Feeding Trial	Concentrations	
	Urine ($mg \cdot ml^{-1}$)	Feces (% Dry Fecal Wt.)
Control	39.7 ± 11.6 (15)	2.8 ± 0.5 (15)
4-Day Gorge-Fast		
Gorging	37.6 ± 13.0 (20)	3.1 ± 0.4 (10)
Fasting	28.6 ± 10.5 (20)	5.3 ± 2.1 (10)
8-Day Gorge-Fast		
Gorging	32.1 ± 11.7 (9)	3.0 ± 0.9 (10)
Fasting	25.6 ± 7.9 (10)	7.1 ± 1.6 (8)
	Total Losses	
	Urine ($mg \cdot kg^{-1} \cdot d^{-1}$)	Feces ($mg \cdot kg^{-1} \cdot d^{-1}$)
Control	856 ± 108 (15)	87 ± 42 (15)
4-Day Gorge-Fast		
Gorging	1030 ± 275 (20)	203 ± 40 (10)
Fasting	339 ± 161 (20)	34 ± 25 (10)
8-Day Gorge-Fast		
Gorging	1216 ± 380 (9)	238 ± 114 (10)
Fasting	214 ± 67 (10)	25 ± 10 (8)

[a] During the Control, wolves were fed a weight-maintaining ration daily; during the 4-Day Gorge-Fast trial, wolves were fed double rations for two days (Gorging) and fasted for two days (Fasting); during the 8-Day Gorge-Fast trial, wolves were fed double rations for four days (Gorging) and fasted for four days (Fasting). Data are given as mean ± standard deviation (sample size). Results of statistical comparisons are listed in Table 2.17.

TABLE 2.17. Statistical comparisons (analysis of variance) of nitrogen parameters, balances, blood concentrations and body temperatures for captive wolves alternately gorged and fasted or fed daily[a] during winter.

	Trials Compared							
Parameter	C v. 4D G	C v. 4D F	C v. 8D G	C v. 8D F	4D G v. 8D G	4D F v. 8D F	4D G v. 4D F	8D G v. 8D F
Nitrogen Concentrations								
Urine	NS	*(4)	NS	*(4)	NS	*(3)	*	NS
Feces	NS	NS(3)	NS	*	*	NS	*	*
Total Daily Nitrogen Losses								
Urine	*	*	*	*	NS	*	*	*
Feces	*	*	*(3)	*	NS(4)	NS	*(4)	*(3)
Balances								
Sodium	*	NS(4)	NS(4)	NS	*	NS	*	NS
Potassium	*	*	NS	NS	NS	NS	*	NS
Nitrogen	*	*	*	*	NS	*	*	*
Blood Concentration								
Total Protein	NS	*	NS	*	NS	NS	*	NS
Hematocrit	NS(4)	NS(4)	NS(4)	*	NS	NS	NS	NS
Body Temperature	*	*(3)	NS	*(3)	NS(4)	NS(3)	NS	*

[a]During the Control (C), wolves were fed a weight-maintaining diet daily; during the 4-Day Gorge-Fast trial (4D), wolves were fed twice the maintenance diet for two days (4D G) and fasted for two days (4D F); during the 8-Day Gorge-Fast trial (8D), wolves were fed twice the maintenance diet for four days (8D G) and fasted for four days (8D F). Asterisk (*) indicates statistical significance at $P = 0.05$; NS indicates lack of statistical significance at $P = 0.05$. If statistical significance differed among the five wolves, results for majority are shown, followed by number of wolves in parentheses. Data compared in this table are listed in Tables 2.16, 2.18, 2.19 and 2.20.

TABLE 2.18. Electrolyte and nitrogen balances of captive wolves alternately gorged and fasted or fed daily[a] during winter.

Feeding Trial	Sodium Balance ($mg \cdot kg^{-1} \cdot d^{-1}$)	Potassium Balance ($mg \cdot kg^{-1} \cdot d^{-1}$)	Nitrogen Balance ($mg \cdot kg^{-1} \cdot d^{-1}$)
Control	- 6.9 ± 15.0 (15)	- 19.5 ± 10.5 (15)	60 ± 131 (15)
4-Day Gorge-Fast			
Gorging	46.4 ± 37.3 (10)	18.1 ± 47.9 (10)	753 ± 312 (10)
Fasting	- 26.7 ± 15.2 (10)	- 38.3 ± 23.9 (10)	- 413 ± 206 (10)
8-Day Gorge-Fast			
Gorging	9.6 ± 37.4 (10)	- 3.6 ± 54.7 (10)	674 ± 537 (10)
Fasting	- 14.9 ± 10.4 (8)	- 20.9 ± 5.8 (8)	- 235 ± 71 (10)

[a] During the Control, wolves were fed a weight-maintaining ration daily; during the 4-Day Gorge-Fast trial, wolves were fed double rations for two days (Gorging) and fasted for two days (Fasting); during the 8-Day Gorge-Fast trial, wolves were fed double rations for four days (Gorging) and fasted for four days (Fasting). Data are given as mean ± standard deviation (sample size). Results of statistical comparisons are listed in Table 2.17.

Serum Total Protein and Hematocrit

Mean serum total protein concentration declined from gorging to fasting, but there was no tendency for change in hematocrit (Tables 2.18 and 2.19).

There was an overall increase in mean serum total protein concentration during the 8-day gorge-fast trial but not during the 4-day gorge-fast trial. There was no evidence of overall hematocrit change in either trial.

Body Temperature

There were two notable differences in mean body temperature (Table 2.20). Both fasting means were significantly lower than the control mean. The fasting mean was significantly lower than the gorging mean during the 8-day gorge-fast trial (Table 2.17).

Ambient Temperature

The mean ambient temperatures at approximately 7:00 A.M. for the control trial, 4-day gorge-fast trial and 8-day gorge-fast trial were -11.2 ± 8.0C, -12.2 ± 3.6C and -24.9 ± 13.7C respectively.

TABLE 2.19. Serum total protein concentrations and hematocrits of captive wolves alternately gorged and fasted or fed daily[a] during winter.

Feeding Trial	Total Protein ($g \cdot dl^{-1}$)	Hematocrit (%)
Control	6.7 ± 0.3 (20)	47 ± 1 (20)
4-Day Gorge-Fast		
Gorging	7.1 ± 0.9 (20)	48 ± 3 (20)
Fasting	6.2 ± 0.8 (20)	47 ± 2 (20)
8-Day Gorge-Fast		
Gorging	7.1 ± 1.1 (10)	48 ± 2 (10)
Fasting	6.3 ± 0.4 (10)	48 ± 1 (10)

[a] During the Control, wolves were fed a weight-maintaining ration daily; during the 4-Day Gorge-Fast trial, wolves were fed double rations for two days (Gorging) and fasted for two days (Fasting); during the 8-Day Gorge-Fast trial, wolves were fed double rations for four days (Gorging) and fasted for four days (Fasting). Data are given as mean ± standard deviation (sample size). Results of statistical comparisons are listed in Table 2.17.

TABLE 2.20. Body temperatures of captive wolves alternately gorged and fasted or fed daily[a] during winter.

Feeding Trial	Body Temperature (C)
Control	37.9 ± 0.4 (120)
4-Day Gorge-Fast	
Gorging	37.7 ± 0.4 (237)
Fasting	37.7 ± 0.4 (240)
8-Day Gorge-Fast	
Gorging	37.8 ± 0.4 (80)
Fasting	37.7 ± 0.4 (80)

[a] During the Control, wolves were fed a weight-maintaining ration daily; during the 4-Day Gorge-Fast trial, wolves were fed double rations for two days (Gorging) and fasted for two days (Fasting); during the 8-Day Gorge-Fast trial, wolves were fed double rations for four days (Gorging) and fasted for four days (Fasting). Body temperatures were measured hourly for 24 hours in the Control, hourly for 96 hours in the 4-Day Gorge-Fast trial and every 6 hours throughout the 8-Day Gorge-Fast trial. Data are given as mean ± standard deviation (sample size). Results of statistical comparisons are listed in Table 2.17.

DISCUSSION

Comparison of Gorging, Fasting and Daily Eating

There were strong similarities and differences between the findings in Chapter 1 and those in the present experiment. Changes in total body water and determinants of water transfer rate were similar in both studies. The primary difference between the two studies was water loss reduction in undernutrition versus water loss enhancement[2] in fasting. The enhanced fasting water loss was indirect evidence that snow intake was not suppressed.

Water Transfer Rate

All parameters which were highly correlated with WTR in Chapter 1 (Table 1.5) were directly related to WTR in the gorging-fasting experiment (Tables 2.7, 2.14, 2.15 and 2.16). When food intake ranged from half to twice maintenance (Chapter 1), WTR was highly correlated with dry matter intake, total urine electrolyte and nitrogen losses, total electrolyte and nitrogen outputs and total fecal energy loss. Similarly, these parameters and WTR were all highest in gorging, lower during daily maintenance-level eating and lowest during fasting. Thus, the WTR differences among the control trial, gorging and fasting are attributed to differences in intakes and outputs of dry matter, electrolytes, nitrogen and energy.

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[2]See Enhanced Urinary Water Loss and Enhanced Fecal Water Loss in **Explanation of Terms**, page xviii.

Mean WTR, snow intake, fecal water loss, urine water loss and evaporative water loss all remained above zero during fasting. Despite the lack of food, osmotically active metabolic waste products (e.g. electrolytes and nitrogen) were produced and eliminated. The osmotic action of these products during elimination maintained WTR, snow intake, urine water and fecal water above zero. Mean snow intake, urine water loss and evaporative water loss during fasting (Table 2.3) were similar to values predicted by the linear equations of Chapter 1 (Table 1.3). The predicted WTR for zero dry matter intake (Table 1.3) was between the mean fasting WTRs for the 4- and 8-day gorge-fast trials (Table 2.7).

Air and ingested snow at temperatures less than 0C did not cause fasting wolves to consume less free water than did fasting dogs having considerably warmer drinking water and ambient temperature air. Snow intake by the fasted wolves was actually greater than or equal to water intake of fasted dogs. Laboratory dogs fasted for less than a week voluntarily drank daily water quantities approximating the snow intake of the fasted wolves, which were also fasted for less than a week (Cizek 1959). In other experiments (Prentiss *et al.* 1959), fasted dogs drank approximately half those amounts.

Total Body Water and Lean Tissue Hydration

There was indirect evidence of fasting net TBW loss by osmotic drag. During fasting, body weight was lost and electrolyte and nitrogen balances were less than during gorging or the control trial. During undernutrition (Chapter 1), weight loss and negative electrolyte and nitrogen balances

were accompanied by TBW losses, so it appears that TBW was also lost during fasting.

Most of the fasting osmotic TBW loss was urinary. Fasting urine water and electrolyte losses exceeded fasting fecal water and electrolyte losses (Tables 2.3, 2.14 and 2.15). Similarly, during undernutrition (Chapter 1), osmotic water loss was greater in the urine than in the feces.

In contrast to Chapter 1 findings, the fasting net TBW loss was accompanied by a net increase in tissue hydration, compared to gorging. In Chapter 1, net TBW loss was accompanied by dehydration during undernutrition.

In the present experiment, there was indirect evidence of dehydration during early gorging. Most individual serum total protein concentrations and serum osmolalities were highest in early gorging. High serum total protein and osmolality occur during dehydration (Grauer and Grauer 1983). Serum total protein concentration and serum osmolality declined later in gorging and remained low throughout fasting, indicating hydration recovery (Table 2.21).

Dehydration in early gorging was attributed to inadequate access to snow. During gorging, snow was only offered for two hours daily while the daily food ration was given in four or more divided portions. Thus, on the first gorging day, snow was offered and removed before the wolves ate the bulk of the daily dry matter. By the second day, when snow was again offered, the wolves had dehydrated because of the large quantity of dry matter ingested. The greatest snow intakes (Table 2.1) were on the second

TABLE 2.21. Daily mean serum total protein concentrations and serum osmolalities of captive wolves alternately gorged and fasted[a] during winter.

Feeding Trial & Day	Total Protein ($g \cdot dl^{-1}$)	Osmolality ($mOsm \cdot kg^{-1}$)
4-Day Gorge-Fast Trial		
Day 1	7.6 ± 1.1 (10)	340 ± 18 (10)
Day 2	6.6 ± 0.4 (10)	309 ± 10 (10)
Day 3	6.3 ± 0.6 (10)	298 ± 5 (10)
Day 4	6.2 ± 1.0 (10)	302 ± 12 (25)
8-Day Gorge-Fast Trial		
Day 2	8.0 ± 0.6 (5)	323 ± 12 (5)
Day 4	6.1 ± 0.5 (5)	309 ± 6 (5)
Day 6	6.7 ± 0.3 (5)	303 ± 4 (5)
Day 8	6.0 ± 0.2 (5)	308 ± 6 (15)

[a] During the 4-Day Gorge-Fast Trial, wolves were fed a twice maintenance ration for two days and fasted for two days; during the 8-Day Gorge-Fast trial, wolves were fed a twice maintenance ration for four days and fasted for four days. Data are given as mean ± standard deviation (sample size). Individual daily values followed the same pattern as the daily means for total protein in 7 of 10 cases in the 4-Day Gorge-Fast trial and 5 of 5 cases in the 8-Day Gorge-Fast trial. Individual daily values followed the same pattern as the daily means for osmolality in 9 of 10 cases in the 4-Day Gorge-Fast trial and 5 of 5 cases in the 8-Day Gorge-Fast trial.

gorging day in both gorge-fast trials and represented initiation of rehydration. If there were a physiological dehydration stimulus during fasting as there may have been during undernutrition (Chapter 1), it was masked by the experimentally induced dehydration of gorging.

Lack of Water Conservation During Fasting

Urine and fecal water concentrations indicated that water conservation was less during fasting than during gorging or the control trial. Urine osmolality was least during fasting, and fecal water content was greatest.

In contrast, water conservation by kidneys and gut was not less during undernutrition than during maintenance (Chapter 1). Urine osmolality did not decline when the wolves were undernourished. Fecal water content during undernutrition was less than during maintenance, indicating that water conservation by the gut was actually higher during undernutrition than during maintenance.

Lower urine osmolality during fasting could be attributed to decreased antidiuretic hormone (ADH) levels or activity. Rats housed at 5C had greater urine output and lower urine concentration than when kept at room temperature (Greenleaf and Fregly 1982). The study concluded that the increased urine output and decreased urine concentration could have resulted from decreased ADH and/or inability to respond to ADH. Other studies have shown that glucocorticoids (Gaunt *et al.* 1957) or catecholamines (Klein *et al.* 1971) administered with ADH will depress the renal response to ADH. Both hormones were elevated in cold-exposed

rats (Munday and Blane 1960, Leduc 1961, Straw and Fregly 1967), and plasma catecholamines and sympathetic activity in dogs were increased during fasting (Kozlowski *et al.* 1983). Thus, fasting and/or low ambient temperatures may have stimulated a glucocorticoid- and/or catecholamine-induced decline in ADH activity and, subsequently, urine osmolality in the wolves (Figure 2.3). The selective advantage (if any) of such a response is unknown.

The increased fasting fecal water content (Table 2.4) was attributed to increased osmotic activity of the fecal dry matter. The fecal dry matter concentrations of osmotically active substances - sodium, potassium and nitrogen - were greatest during fasting (Tables 2.15 and 2.16). The source of the increased osmotic fraction in the fecal dry matter is not clear.

Result of Testing the Hypothesis

The above findings are indirect evidence that snow intake was not suppressed. Tissue water content was greater during fasting than during gorging, and water conservation was less efficient during fasting than during gorging or the control trial. The enhanced urinary and fecal water losses were indirect evidence that snow intake was enhanced[3]: since dehydration did not appear to accompany these enhanced water losses, snow intake must also have been enhanced.

The hypothesis could not be reliably tested with the tissue hydration data but was tested with the water conservation data. As explained above,

[3]See Enhanced Snow Intake in **Explanation of Terms**, page xviii.

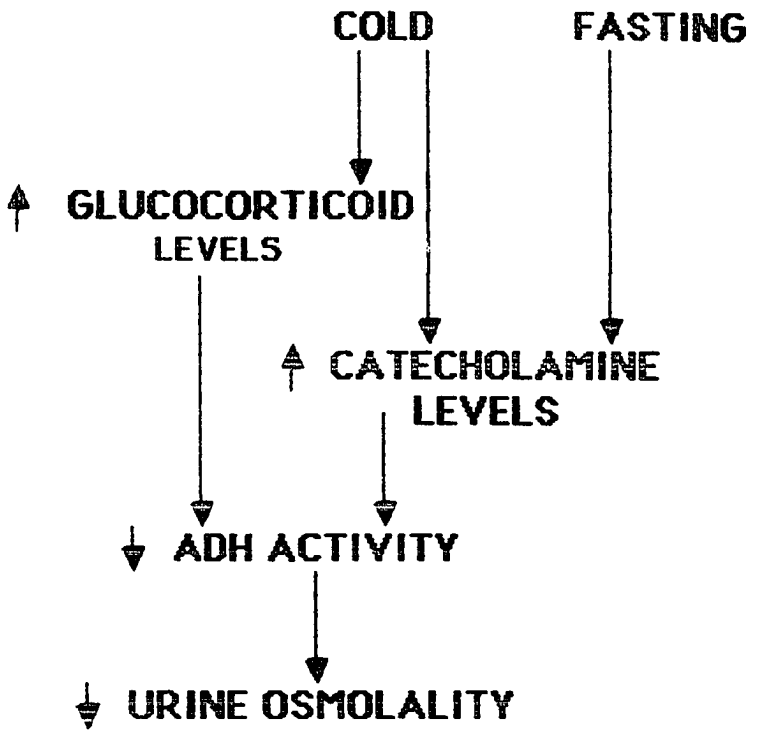

Figure 2.3. Possible paths by which a decline in urine osmolality may have resulted from low air temperatures and/or fasting in captive wolves during winter.

experimental design was the apparent cause of the decrease in tissue hydration during gorging relative to that during fasting; there were not sufficient data to compare tissue water content of fasting and the control trial. The water conservation data were useful because fasting could be compared to the control trial; urine and fecal water contents were greater during fasting than during the control trial, indicating less water conservation during fasting. The water conservation data refute the hypothesis that during winter, wolves reduce energy expenditure by suppressing snow intake when energy balance is negative.

Comparison of Alternate Gorging-Fasting with Daily Eating

Total Body Water, Hydration and Body Water Subcompartments

Total body water volume and tissue water content were better conserved during alternate gorging-fasting than during daily feeding but differed little between the two gorge-fast trials. There were overall TBW losses in all three trials, but the greatest total losses and the greatest rates of loss ($l \cdot d^{-1}$) were in the control trial (Table 2.6). Lean tissue dehydrated during each trial (Table 2.22), but the wolves were less dehydrated after sixteen days of alternate gorging and fasting than after seven days of daily feeding (Table 2.22). The TBW losses (Table 2.7) and the frequency of dehydration among the five wolves (Table 2.22) were similar in the two gorge-fast trials.

The distribution of overall TBW loss among body water subcompartments differed for each trial (Table 2.6). During the control trial, water was lost from ECF and ICF, and the ECF loss was entirely ISF.

TABLE 2.22. Changes in body weight, body water and body solids of captive wolves after sixteen days of alternate gorging-fasting and after seven days of eating a weight-maintaining ration[a] during winter.

Trial	Wolf	ΔBW[b] (kg)	ΔTBW/ΔLean[c] ($l \cdot kg^{-1}$)	ΔTBW/ ΔP[d] ($l \cdot kg^{-1}$)	ΔF[e] (kg)	ΔP[f] (kg)	ΔO[g] (kg)
C	Rusty	+ 1.4	↓	↓	+ 1.7	+ 0.1	0.0
C	Butch	+ 0.7	↓	↓	+ 2.0	+ 0.3	0.0
C	Mix	+ 0.4	↓	↓	+ 0.7	+ 0.1	0.0
C	Frosty	+ 2.2	↓	↓	+ 2.8	+ 0.1	- 0.1
C	Mr. Brown	- 0.1	0.734 NC	↓	+ 1.4	0.0	- 0.4
4-D	Rusty	+ 1.7	↑	0.15 ↓	+ 1.5	+ 1.2	- 1.2
4-D	Butch	+ 1.9	0.719 ↑	↓	+ 2.2	+ 0.9	- 1.0
4-D	Mix	+ 0.3	↓	↓	+ 0.3	+ 0.4	- 0.4
4-D	Frosty	- 1.0	0.735 NC	0.50 ↓	- 0.7	- 0.5	+ 0.4
4-D	Mr. Brown	+ 0.4	↑	0.03 ↓	+ 0.4	+ 0.8	- 0.8
8-D	Rusty	+ 0.6	↑	↑	+ 0.4	- 0.2	+ 0.1
8-D	Butch	+ 0.6	0.667 ↑	↓	+ 0.8	+ 1.3	- 1.4
8-D	Mix	+ 0.3	↓	↓	+ 0.4	+ 0.6	- 0.6
8-D	Frosty	- 1.7	0.721 ↑	↓	- 0.7	+ 1.7	- 2.0
8-D	Mr. Brown	- 0.3	0.780 ↓	↓	+ 0.1	+ 1.4	- 1.5

[a] During the Control trial (C), wolves were fed a weight-maintaining ration daily; during the 4-Day Gorge-Fast trial (4-D), wolves were fed double rations for two days and fasted for two days; during the 8-Day Gorge-Fast trial (8-D), wolves were fed double rations for four days and fasted for four days. [b]ΔBW is change in body weight; [c]ΔTBW/ΔLean is ratio of change in total body water to change in lean mass; [d]ΔTBW/ΔP is ratio of change in total body water to change in body protein content; [e]ΔF is change in body fat; [f]ΔP is change in body protein; [g]ΔO is change in nonfat nonprotein body solids. Arrows (↑,↓) show direction of change in lean tissue water content; NC = no change. Where ΔTBW and either ΔP or change in nonfat solids differed in direction of change, no numeric value for ratio is given; ↑ indicates TBW increase, nonfat solids or dry protein decline; ↓ indicates TBW decline and nonfat solids or dry protein increase. Based on total number of individual hydration increases and decreases in each trial, lean tissue dehydration occurred more frequently in the Control trial than in either Gorge-Fast trial.

During the 4-day gorge-fast trial, the overall water loss was primarily from ICF. During the 8-day gorge-fast trial, the overall water loss was primarily from ECF, and the ECF loss was from plasma as well as ISF. There are insufficient data to explain the differences among trials.

The distribution of overall TBW loss during each trial differed from the distribution of TBW loss in laboratory dogs. When dogs were dehydrated by water deprivation, water was lost primarily from ECF and ISF (Hamilton and Schwartz 1935, Elkinton and Taffel 1942, DeBoer 1946). The differences between the canine studies and the wolf study could be due to the greater dehydration of the dogs.

Water Transfer Rate, Water Intakes, and Water Outputs

Alternate gorging-fasting produced indirect evidence of increased WTR in the wolves as it did in sheep (Watson *et al.* 1975). In both experiments, there were increases in water intakes and outputs, suggesting increased WTR with alternate gorging-fasting. Overall snow intake and fecal water loss by the wolves were greater during the gorge-fast trials than during the control trial (Table 2.23). Sheep gorged and fasted in a seven-day cycle had higher total water intake and urine output than sheep fed the same quantity of food in seven equal daily portions (Watson *et al.* 1975). In the wolf study, the 4-day gorge-fast trial had greater total urine water loss, total fecal water loss, total snow intake and daily fractional water turnover rate than did the control trial (Table 2.23). This comparison of the 4-day gorge-fast and control trials is consistent with the sheep study. Comparison of the 8-day gorge-fast trial

TABLE 2.23. Comparison of individual overall mean daily fractional water turnover rates, water intakes and water outputs between a daily feeding trial and two gorging-fasting trials[a] for captive wolves during winter.

Parameter	Trials Compared: Control & 4-Day Gorge-Fast — Number of Increases: Number of Decreases or Number with No Change	Control & 8-Day Gorge-Fast — Number of Increases: Number of Decreases
Urine Water	**3:1**	2:2
Fecal Water	**3:1**	**4:0**
Evaporative Water	2:2	1:3
Snow Water	**4:1**	**3:2**
Fractional Turnover Rate	**4:1**	2:3

[a]During the Control trial, wolves were fed a weight-maintaining ration daily; during the 4-Day Gorge-Fast trial, wolves were fed a twice maintenance ration for two days and fasted for two days; during the 8-Day Gorge-Fast trial, wolves were fed a twice maintenance ration for four days and fasted for four days. Each number represents number of wolves having an increase, decrease or no change in water parameter between two trials. Where increases outnumbered decreases or lack of change, ratio is bold face and enlarged for contrast.

and the control trial was similar but not as consistent. In the 8-day gorge-fast trial, only total fecal water loss and total snow intake were greater than in the control trial.

These data revealed that snow intake was enhanced rather than suppressed. In both gorge-fast trials, most individual overall snow intakes were increased, compared to the control trial. Since overall gorge-fast snow intakes were higher than in the control trial and overall dry matter was unchanged, the overall snow intake-dry matter intake ratios were higher during the gorge-fast trials than during the control trial.

The snow intake-energy expenditure ratios could not be compared among trials because the calculated control energy expenditure values may be incorrect. The calculated mean daily energy expenditure of the control trial was only about 80% of the calculated basal metabolic rate and therefore totally unreasonable. The calculated mean overall daily gorge-fast energy expenditures were about three times the calculated basal metabolic rate and therefore reasonable. The reason for the discrepancy is unknown.

Energy Balance of Alternate Gorging-Fasting Versus Daily Eating

It was not possible to clearly document decreased energy balance in alternate gorging-fasting compared to the control trial. Overall energy balance appeared to be lower during alternate gorging-fasting than during the control: calculated overall daily gorge-fast energy expenditure was greater than calculated control daily energy expenditure, while mean daily

food intake in all trials was the same. Unfortunately, the unrealistic estimate for control daily energy expenditure made such a conclusion questionable. In addition, comparison with Chapter 1 data suggests approximately equal overall daily energy balances for the gorge-fast trials and for daily maintenance feeding: mean daily energy expenditures for the gorge-fast trials were nearly identical to the mean daily energy expenditure for daily maintenance intake (Chapter 1).

It is possible that wolves have evolved to increase absorptive efficiency when gorging so that there is no increase in total heat loss and no decrease in energy balance relative to eating a daily maintenance ration. Calculations based on Chapter 1 data support this possibility: individual energy digestibilities increased from maintenance feeding to twice maintenance feeding.

Result of Testing the Hypothesis

Snow intake suppression did not accompany alternate gorging-fasting. In fact, there was enhanced overall gorge-fast snow intake as indicated by the greater overall tissue hydration and larger overall snow intake-dry matter intake ratios than in the control trial.

The conflicting energy balance data precluded confirmation of negative energy balance during the gorge-fast trials. Had overall negative energy balance been confirmed, the lack of overall snow intake suppression would have refuted the hypothesis that, during winter, wolves reduce energy expenditure by suppressing snow intake when energy balance is negative. The hypothesis will remain untested by this phase of the

experiment until the energy balances of alternate gorging-fasting and daily feeding are more accurately determined for the conditions in this experiment.

CONCLUSIONS

1. Water transfer rate was directly related to dry matter intake, total urine electrolyte and nitrogen losses, total electrolyte output and total nitrogen output of captive wolves during winter. These determinants of water transfer rate varied in the wolves due to gorging, fasting and consuming a daily maintenance ration. Water transfer continued during fasting, as predicted by the Chapter 1 data.

2. During fasting, the wolves had a net body water loss but a net increase in lean tissue hydration. In contrast, undernourished wolves (Chapter 1) had a net body water loss and a net decrease in lean tissue hydration. Experimental design in the gorge-fast trials may have caused the difference.

3. There was less water conservation by the kidneys and gut during fasting than during gorging or daily maintenance feeding. In contrast, renal and intestinal water conservation was not less during undernutrition than during maintenance (Chapter 1). The decline in water conservation during fasting was indirect evidence of enhanced snow intake; this finding refutes the hypothesis that during winter, wolves reduce energy expenditure by suppressing snow intake when energy balance is negative.

4. The overall conditions found during alternate gorging-fasting did not indicate snow intake suppression. Greater overall tissue hydration and greater overall snow intake-dry matter intake ratios during alternate gorging-fasting indicated greater overall snow intake than during daily

maintenance feeding. The hypothesis could not be tested because a decline in overall energy balance from daily maintenance feeding to alternate gorging-fasting could not be unequivocally documented.

Chapter 3

Water Metabolism of Captive Wolves in Winter. IV. Effects of Exercise

INTRODUCTION

The previous two chapters addressed water metabolism of captive wolves when energy balance was varied by changing food intake. Water transfer rate and snow intake were directly related to dry matter intake when food intake varied from undernutrition to overnutrition and when wolves gorged, fasted and consumed a daily maintenance ration. There was no consistent relationship between snow intake suppression and negative energy balance: snow intake was suppressed during undernutrition but not during fasting.

This chapter addresses water metabolism of captive wolves when daily food intake was constant and energy balance was varied by changing the daily amount of exercise. Energy balance was expected to decline when daily energy expenditure was increased and food intake was unchanged.

Wolves engage in a great deal of physical activity. They may spend 20% to 30% of their time hunting (Mech 1970), covering distances of up to 70 km in 24 hours (Burkholder 1959). Killing prey requires chasing and attacking, which are usually successful less than 50% of the time (Mech 1970, Zimen 1981). Other activities include mating, playing and struggles for rank.

Exercise has direct short-term effects on water metabolism in canids The heat of exercise was dissipated primarily by evaporation (panting) in domestic dogs and African hunting dogs (Taylor *et al.* 1971, Taylor 1974). The water lost by evaporation in exercising dogs decreased plasma volume (O'Connor 1975) and was accurately replentished by drinking (Greenleaf *et al.* 1976).

There have been no studies addressing long-term effects of exercise on water metabolism in canids. It is not known how resting total body water volume and the body water subcompartments of canids are influenced by changes in daily exercise. In humans, exercise training increased resting plasma volume (Holmgren *et al.* 1964). This increased post-training plasma volume was associated with increased cardiac output and work performance during exercise. The effects of exercise on water metabolism during negative energy balance, in cold ambient air and with snow as the only free water source have not been studied in any species.

This experiment was designed to provide information on how winter water metabolism of wolves is influenced by changes in total daily amount of exercise. It tested the hypothesis that wolves reduce energy expenditure by suppressing snow intake when in negative energy balance during winter. The objective was to determine the effects on winter water metabolism of captive wolves when total daily exercise increased and daily food intake remained unchanged.

METHODS

Experimental Animals

Four adult male wolves were used in this experiment. Three were 4.5 years old, and one was 1.5 years old. Housing and care were as described in Chapter 1.

Experimental Conditions

The experiment consisted of two trials - an eight day control trial (mid-December) and a nine day exercise trial (April). Prior to the exercise trial, the wolves were conditioned in metabolism cages for 13 days. During the last ten days of conditioning, the wolves were trained to trot on a treadmill at a 10% incline. Each wolf ran for 37.5 minutes twice daily with a one minute warm-up and a two minute cool-down at walking speed. There was a 2.0 to 2.5 hour rest between runs. Trotting speed was 9.9 to 11.6 km $\cdot$ hr^{-1} at the start of training and was gradually increased, reaching 11.7 to 13.7 km $\cdot$ hr^{-1} by the start of the exercise trial. The final speed was a fast trot similar to the 10 to 13 km $\cdot$ hr^{-1} normal travelling speed of wild wolves (Stephenson 1978). This speed was used for the exercise trial. The treadmill incline gave 1.2 to 1.4 km $\cdot$ hr^{-1} vertical speed. All daily exercise was done after injections and blood samplings but before feeding. In both trials, a daily maintenance Zu/Preem ration (37.5 g $\cdot$ kg^{-1}, Chapter 1) and ad libitum snow were offered to each wolf for two hours every afternoon. The experimental day began when uneaten food and snow were removed.

Body Weight

Body weight was recorded on alternate days in both trials. The weighing procedure was described in Chapter 2.

Body Temperature

Body temperature was monitored by the methods described in Chapter 1. Additionally, each trial had one 24 hour monitoring period during which body temperature was recorded hourly (exercise trial) or every two hours (control trial). Both Mark IV and Mark V transmitters were used.

Ambient Temperature

Ambient temperature was measured as described in Chapter 1.

Urine, Fecal, Blood and Food Analyses

Sample Collection

Urine was collected daily as described in Chapter 1. Daily samples were used for determination of urinary water loss. Samples for determining osmolality, electrolyte concentrations and total daily electrolyte losses were collected on alternate days during the control trial and every day during the exercise trial. Samples for determining total nitrogen loss, nitrogen concentration and energy concentration were collected on alternate days in both trials.

Feces were collected daily as described in Chapter 1, but no 24 hour collections were made. Daily samples were used for measurement of fecal

water concentration and daily fecal water loss. Samples for electrolyte, nitrogen and energy concentrations and for total electrolyte, nitrogen and energy losses were collected on alternate days of both trials.

Blood sampling methods were described in Chapter 1. Parameters measured were serum electrolyte (sodium, potassium, chloride) concentrations, serum osmolality, serum glucose concentration, serum triglyceride concentration, plasma free fatty acid concentration, serum total protein concentration, blood urea nitrogen concentration and hematocrit. Blood samples for these parameters were collected on alternate days in both trials.

Sample Analyses

Urine specific gravity was measured with a refractometer. Serum glucose and blood urea nitrogen concentrations were determined with a clinical spectrophotometer (Serometer 360, Mallincrodt, Inc., Bohemia, NY). Serum triglyceride concentrations were determined with an automated procedure at the Walter Reed Army Institute of Research (Washington, DC). Plasma free fatty acid concentrations were determined by a commercial laboratory (Reference Laboratory, Newbury Park, CA). All other analytical methods were described in Chapter 1.

Water Intake and Output

Water intake determinations (including oxidative water) were as described in Chapter 1. Water loss determinations were similar to those

in Chapter 1; the only exception was that urine water content was calculated with Equation 2.1.

Total Body Water, Water Transfer Rate and Body Fat

Tritiated water (TOH) was used to measure total body water (TBW) and water transfer rate (WTR). Each TOH injection was by cephalic venapuncture and contained approximately 500 µCi of tritium in 1.0 ml. All four wolves were injected on the first and last day of each trial. The final injections of TOH for each trial were mixed with 1.0 ml of radiosulfate.

Initial and final TBWs for each trial were calculated from the mean specific activities of three samples taken at variable intervals 1.5 to 4.0 hours after injection. Sample processing was described in Chapter 1.

Water transfer rate was calculated as described in Chapter 1. Blood samples for WTR determination were collected on alternate days of both trials. Fractional daily body water turnover rate (k) was calculated with Equation 1.2.

Body fat was calculated from body water percent according to Equation 1.4.

Body Water Subcompartments

Extracellular Fluid Space

Functional extracellular fluid space (ECF) was estimated as the radiosulfate space on the last day of each trial. Each wolf was injected intravenously with approximately 200 µCi radiosulfate in 1.0 ml mixed

with 1.0 ml TOH. Sampling and sample processing were described in Chapter 2.

Plasma Volume

Plasma volume (PV) was estimated by intravenous injection of radioiodinated (^{125}I) human serum albumin (RIHSA) and calculation of the RIHSA space. Injection, sampling, sample processing and PV calculation were described in Chapter 2. Plasma Volume measurements were made on the same days as ECF measurements.

Intracellular and Interstitial Fluid Volumes

Intracellular fluid volume (ICF) was calculated by subtraction of ECF from TBW. Interstitial fluid volume (ISF) was calculated by subtraction of PV from ECF.

Calculations

Total changes in body protein (ΔP, kg), body fat (ΔF, kg), body nonfat nonprotein solids (ΔO, kg) and lean body mass (ΔLean, kg) were calculated from Equations 1.4 through 1.9.

The ratios ΔTBW/ΔLean and ΔTBW/ΔP were used to evaluate changes in tissue hydration. Calculation and use of both ratios were described in Chapter 1.

Calculation of daily energy expenditure and the water transfer rate-energy expenditure ratio were described in Chapter 1.

Statistical Analyses

Data were compared by analysis of variance (ANOVA) at P = 0.05. Data were first compared by day within each trial. If data for any days were significantly different from those of other days, the trial data were partitioned into subsections for further comparisons.

Next, ANOVA was used to detect significant first order interaction between wolf and trial. Where such interaction occurred, the data were partitioned by wolf for further comparison.

Lastly, one way ANOVA was used to compare control trial data with exercise trial data. Data were partitioned by subsection and/or wolf where appropriate.

RESULTS

Body Weight

Fractional body weight did not change significantly during the control trial. Fractional body weight declined significantly during the exercise trial, by 0.2% $\cdot$ d^{-1} (linear regression, $n = 20$, $r = 0.676$, $P < 0.005$).

Water Transfer Rate, Water Intakes and Water Outputs

Water transfer rate and most intakes and outputs were significantly greater during the exercise trial (Table 3.1). Only daily oxidative and urine water losses did not differ between trials.

Fecal water content was significantly greater during the exercise trial than during the control trial (Table 3.1).

Total Body Water and Body Fat

All individual TBWs and total body water percentages (TBW%s) increased during both trials (Tables 3.2 and 3.3). The exercise trial increases were noticeably less than the control trial increases. Additionally, all individual TBWs and TBW%s were higher after the exercise trial than at the end of the control trial.

Body Water Subcompartments

All body water subcompartments changed from the control trial to the exercise trial. All individual extracellular fluid volumes increased in total volume (ECF), as percent of body weight (ECF%) and as percent of lean

TABLE 3.1. Water intake and output parameters of captive wolves confined to cages or exercised daily[a] during winter.

Parameter	Trial		ANOVA
	Control	Exercise	
Water Transfer Rate ($ml \cdot kg^{-1} \cdot d^{-1}$)	33.8 ± 2.7 (4)	60.1 ± 2.4 (4)	*
Fractional Daily Turnover Rate (d^{-1})	0.060 ± 0.003 (4)	0.100 ± 0.005 (4)	*
Water Intake ($ml \cdot kg^{-1} \cdot d^{-1}$)			
Snow Water	9.4 ± 5.7 (28)	28.1 ± 8.0 (32)	*
Food Water	20.4 ± 4.0 (28)	21.5 ± 0.2 (32)	*
Metabolic Water[b]	3.3 ± 5.3 (28)	10.5 ± 7.6 (32)	*
Oxidative Water[b]	12.5 ± 2.9 (4)	12.2 ± 0.6 (4)	NS
Water Output ($ml \cdot kg^{-1} \cdot d^{-1}$)			
Urine Water	14.4 ± 2.9 (28)	15.2 ± 3.2 (32)	NS
Fecal Water	5.8 ± 1.7 (28)	6.6 ± 1.0 (32)	*
Evaporative Water[b]	13.6 ± 3.8 (28)	38.3 ± 3.7 (32)	*
Fecal Water Content (%)	62.6 ± 1.8 (28)	64.0 ± 1.7 (36)	*

[a] During the Control trial, wolves were confined to cages; during the Exercise trial, wolves exercised on a treadmill daily. [b]Metabolic water and evaporative water were calculated by subtraction; oxidative water was calculated from the algebraic sum of oxidative water equivalents of nutrients catabolized and body tissue changes. ANOVA represents results of statistical comparison between trials: * indicates significant difference at $P = 0.05$; NS represents lack of significance at $P = 0.05$. Data are given as mean ± standard deviation (sample size).

TABLE 3.2. Total body water and body fat of captive wolves confined to cages or exercised daily[a] during winter.

Wolf	Trial	Initial Trial Values			Final Trial Values		
		TBW[b] (l)	TBW%[c] (%)	% Fat[d] (%)	TBW (l)	TBW% (%)	% Fat (%)
Moonlite	Control	27.55	54.3	25.8	28.78 ↑	55.9 ↑	23.7 ↓
Nanook	Control	25.64	54.8	25.2	26.44 ↑	55.4 ↑	24.3 ↓
Siqiniq	Control	24.54	60.0	18.0	25.92 ↑	63.4 ↑	13.4 ↓
Uncle Greg	Control	25.78	55.1	24.7	27.41 ↑	58.1 ↑	20.7 ↓
mean ± s.d.			56.1 ± 2.7	23.4 ± 3.6		58.2 ± 3.7	20.5 ± 5.0
Moonlite	Exercise	29.57	57.9	20.9	30.33 ↑ E↑	59.7 ↑ E↑	18.4 ↓ E↓
Nanook	Exercise	29.14	58.0	20.7	29.40 ↑ E↑	59.7 ↑ E↑	18.4 ↓ E↓
Siqiniq	Exercise	26.99	61.6	15.8	27.15 ↑ E↑	63.9 ↑ E↑	12.7 ↓ E↓
Uncle Greg	Exercise	28.48	62.2	15.0	28.59 ↑ E↑	63.5 ↑ E↑	13.3 ↓ E↓
mean ± s.d.			59.9 ± 2.3	18.1 ± 3.1		61.7 ± 2.3	15.7 ± 3.2

[a] During the Control trial, wolves were confined to cages; during the Exercise trial, wolves exercised on a treadmill daily. [b]TBW = total body water. [c]TBW% = total body water as percent of body weight. [d]% Fat = body fat as percent of body weight. Arrows not preceded by letter (↑, ↓) indicate direction of change from initial to final measurement within trial. Arrows following letter E (E↑, E↓) indicate change from the Control trial to the end of the Exercise trial.

TABLE 3.3. Changes in total body water of captive wolves confined to cages or exercised daily[a] during winter.

Wolf	Time Span	ΔTBW[b] (l)	ΔTBW%[c] (%)
Moonlite	Control (initial to final)	+ 1.23	+ 1.6
Nanook	Control (initial to final)	+ 0.80	+ 0.6
Siqiniq	Control (initial to final)	+ 1.38	+ 3.4
Uncle Greg	Control (initial to final)	+ 1.63	+ 3.0
Moonlite	Exercise (initial to final)	+ 0.76	+ 1.8
Nanook	Exercise (initial to final)	+ 0.26	+ 1.7
Siqiniq	Exercise (initial to final)	+ 0.16	+ 2.3
Uncle Greg	Exercise (initial to final)	+ 0.11	+ 1.3
Moonlite	End of Control to end of Exercise	+ 0.79	+ 3.8
Nanook	End of Control to end of Exercise	+ 2.70	+ 4.3
Siqiniq	End of Control to end of Exercise	+ 1.07	+ 0.5
Uncle Greg	End of Control to end of Exercise	+ 1.07	+ 5.4

[a] During the Control trial, wolves were confined to cages;during the Exercise trial, wolves exercised on a treadmill daily. [b]ΔTBW = change in total body water. [c]ΔTBW% = change in total body water as percent of body weight.

body weight (ECF % of Lean) (Table 3.4). Most individual intracellular fluid volumes declined in absolute volume (ICF); the numbers of increases and decreases were equal for ICF as percent of body weight (ICF%); and all individual ICFs as percent of lean body weight (ICF % of Lean) declined (Table 3.5). Most individual plasma volumes increased as percent of body weight (PV%); the numbers of individual increases and decreases were equal for changes in absolute volume (PV) and for percent of lean body weight (PV % of Lean) (Table 3.6). Most wolves had increased interstitial fluid volume as absolute volume (ISF), percent of body weight (ISF%) and percent of lean weight (ISF % of Lean) (Table 3.7). In most cases the changes in PV, PV% and PV % of Lean were markedly less than the corresponding changes in the other three water subcompartments.

Electrolytes

Intake

Electrolyte concentrations in the food dry matter were 4.3 ± 0.8 mg potassium · g^{-1} (n = 3) and 4.1 ± 0.7 mg sodium · g^{-1} (n = 3). The respective daily electrolyte intakes were 68.1 and 66.2 mg · kg^{-1}. Electrolyte intake in snow was assumed to be negligible.

Blood Levels

Mean serum sodium concentrations at the beginning and end of the exercise trial (two of five sampling days) were significantly less than the control mean (Table 3.8). Mean serum osmolality was significantly less during the exercise trial than during the control trial (Table 3.8).

TABLE 3.4. Functional extracellular fluid space of captive wolves confined to cages or exercised daily[a] during winter.

Wolf	Control Values			Exercise Values		
	ECF[b] (l)	ECF%[c] (%)	ECF % of Lean[d] (%)	ECF (l)	ECF% (%)	ECF % of Lean (%)
Moonlite	10.27	19.9	26.1	13.22	26.0	31.9
Nanook	8.13	17.0	22.5	10.22	20.8	25.4
Siqiniq	8.95	21.9	25.3	10.36	24.4	27.9
Uncle Greg	9.34	19.8	25.0	10.63	23.6	27.3

Wolf	ΔECF[e] (l)	ΔECF% (%)	ΔECF % of Lean (%)
Moonlite	+ 2.95	+ 6.1	+ 5.8
Nanook	+ 2.09	+ 3.8	+ 2.9
Siqiniq	+ 1.41	+ 2.5	+ 2.6
Uncle Greg	+ 1.29	+ 3.8	+ 2.3

[a] During the Control trial, wolves were kept in cages without exercise; during the Exercise trial, wolves ran on a treadmill daily. [b]ECF = functional extracellular fluid space. [c]ECF% = functional extracellular fluid space as percent of body weight. [d]ECF % of Lean = functional extracellular fluid space as % of lean body weight. [e]Δ indicates change in quantity from end of the Control trial to end of the Exercise trial.

TABLE 3.5. Intracellular fluid space of captive wolves confined to cages or exercised daily[a] during winter.

Wolf	Control Values			Exercise Values		
	ICF[b] (l)	ICF% [c] (%)	ICF % of Lean[d] (%)	ICF (l)	ICF% (%)	ICF % of Lean (%)
Moonlite	18.51	36.0	47.1	17.11	33.7	41.3
Nanook	18.31	38.4	50.7	19.18	38.9	47.7
Siqiniq	16.97	41.5	47.9	16.97	39.5	45.3
Uncle Greg	18.07	38.3	48.3	17.96	39.9	46.1
Wolf	ΔICF[e] (l)	ΔICF% (%)	ΔICF % of Lean (%)			
Moonlite	- 1.40	- 2.3	- 5.8			
Nanook	+ 0.87	+ 0.5	-3.0			
Siqiniq	- 0.18	- 2.0	- 2.6			
Uncle Greg	- 0.11	+ 1.6	- 2.2			

[a] During the Control trial, wolves were kept in cages without exercise; during the Exercise trial, wolves ran on a treadmill daily. [b]ICF = intracellular fluid space. [c]ICF% = intracellular fluid space as percent of body weight. [d]ICF % of Lean = intracellular fluid space as % of lean body weight. [e]Δ indicates change in quantity from end of the Control trial to end of the Exercise trial.

TABLE 3.6. Plasma volume of captive wolves confined to cages or exercised daily[a] during winter.

Wolf	Control Values			Exercise Values		
	PV[b] (l)	PV%[c] (%)	PV % of Lean[d] (%)	PV (l)	PV% (%)	PV % of Lean (%)
Moonlite	3.80	7.4	9.7	3.80	7.5	9.2
Nanook	3.66	7.7	10.1	4.44	9.0	11.1
Siqiniq	3.72	9.1	10.5	3.58	8.4	9.6
Uncle Greg	3.98	8.1	10.6	6.02	13.4	15.4
Wolf	ΔPV[e] (l)	ΔPV% (%)	ΔPV % of Lean (%)			
Moonlite	0.0	+ 0.1	- 0.5			
Nanook	+ 0.78	+ 1.3	+ 1.0			
Siqiniq	- 0.14	- 0.7	- 0.9			
Uncle Greg	+ 2.04	+ 5.0	+ 4.8			

[a] During the Control trial, wolves were kept in cages without exercise; during the Exercise trial, wolves ran on a treadmill daily. [b]PV = plasma volume. [c]PV% = plasma volume as percent of body weight. [d]PV % of Lean = plasma volume as % of lean body weight. [e]Δ indicates change in quantity from end of the Control trial to end of the Exercise trial.

TABLE 3.7. Interstitial fluid space of captive wolves confined to cages or exercised daily[a] during winter.

Wolf	Control Values			Exercise Values		
	ISF[b] (l)	ISF%[c] (%)	ISF % of Lea[d] (%)	ISF (l)	ISF% (%)	ISF % of Lean (%)
Moonlite	6.47	12.5	16.5	9.42	18.5	22.8
Nanook	4.47	9.3	12.4	5.78	11.8	14.4
Siqiniq	5.23	12.8	14.8	6.78	16.0	18.3
Uncle Greg	5.36	11.4	14.3	4.61	10.2	11.8

Wolf	ΔISF[e] (l)	ΔISF% (%)	ΔISF % of Lean (%)
Moonlite	+ 2.95	+ 6.0	+ 6.3
Nanook	+ 1.31	+ 2.5	+ 2.0
Siqiniq	+ 1.55	+ 3.2	+ 3.5
Uncle Greg	- 0.75	- 1.2	- 2.5

[a] During the Control trial, wolves were kept in cages without exercise; during the Exercise trial, wolves ran on a treadmill daily. [b]ISF = interstitial fluid space. [c]ISF% = interstitial fluid space as percent of body weight. [d]ISF % of Lean = interstitial fluid space as % of lean body weight. [e]Δ indicates change in quantity from end of the Control trial to end of the Exercise trial.

TABLE 3.8. Serum electrolyte concentrations and serum osmolalities of captive wolves confined to cages or exercised daily[a] during winter.

Trial	Parameter			
	Sodium ($meq \cdot l^{-1}$)	Potassium ($meq \cdot l^{-1}$)	Chloride ($meq \cdot l^{-1}$)	Osmolality ($mOsm \cdot kg^{-1}$)
Control	146 ± 2 (20)	4.2 ± 0.4 (20)	128 ± 8 (20)	312 ± 13 (20)
Exercise	139 ± 10 (20)	4.0 ± 0.3 (20)	124 ± 9 (20)	303 ± 3 (20)
	*/NS	NS	*/NS	*

[a]During the Control trial, wolves were confined to metabolism cages; during the Exercise trial, wolves were exercised daily on a treadmill. Data are given as mean ± standard deviation (sample size). * indicates significant difference at $P = 0.05$ (ANOVA); NS represents lack of significance at $P = 0.05$; */NS indicates mixed statistical comparisons. For sodium, 2 of 5 days were significantly different; for chloride, 2 of 4 wolves had significant differences.

There were no significant differences in mean serum potassium concentration between trials. Mean serum chloride concentration declined from the control trial to the exercise trial in two wolves but did not change significantly in the other two wolves.

Output

The major finding regarding urine electrolyte losses was a significant decrease in urine osmolality from the control trial to the exercise trial (Table 3.9). There were no significant changes in urine electrolyte concentrations or total daily urine electrolyte losses.

Fecal electrolyte concentrations and total daily losses did not differ between trials (Table 3.10).

Nitrogen

Intake

Nitrogen content of the food dry matter was 62.0 ± 3.6 mg • g^{-1} (n = 3). Daily nitrogen intake for both trials was 1.003 g • kg^{-1}.

Blood Levels

Mean blood urea nitrogen concentration increased significantly from the control trial to the exercise trial (Table 3.11).

Output

The major findings regarding nitrogen output were a significant decrease in mean urine concentration and a significant increase in mean

TABLE 3.9. Urine electrolyte concentrations and osmolalities of captive wolves confined to cages or exercised daily[a] during winter.

Trial	Concentrations			
	Sodium ($meq \cdot l^{-1}$)	Potassium ($meq \cdot l^{-1}$)	Chloride ($meq \cdot l^{-1}$)	Osmolality ($mOsm \cdot kg^{-1}$)
Control	138 ± 23 (20)	122 ± 13 (20)	212 ± 24 (20)	2432 ± 253 (20)
Exercise	141 ± 23 (36)	119 ± 19 (36)	185 ± 64 (20)	2287 ± 252 (36)
	NS	NS	NS	*

Trial	Total Daily Losses		
	Sodium ($mg \cdot kg^{-1} \cdot d^{-1}$)	Potassium ($mg \cdot kg^{-1} \cdot d^{-1}$)	Chloride ($mg \cdot kg^{-1} \cdot d^{-1}$)
Control	55 ± 11 (16)	84 ± 13 (16)	132 ± 24 (16)
Exercise	56 ± 11 (36)	79 ± 16 (36)	122 ± 46 (20)
	*/NS	NS	NS

[a]During the Control trial, wolves were confined to metabolism cages; during the Exercise trial, wolves were exercised daily on a treadmill. Data are given as mean ± standard deviation (sample size). * indicates significant difference at P = 0.05 (ANOVA); NS represents lack of significance at P = 0.05; */NS indicates mixed statistical comparisons. For total sodium loss, 2 of 9 days were significantly different.

TABLE 3.10. Fecal electrolytes of captive wolves confined to cages or exercised daily[a] during winter.

	Concentrations (% of dry fecal weight)		Total Daily Losses ($mg \cdot kg^{-1} \cdot d^{-1}$)	
Trial	Sodium	Potassium	Sodium	Potassium
Control	0.4 ± 0.2 (12)	0.2 ± 0.1 (12)	15 ± 5 (12)	8 ± 2 (12)
Exercise	0.3 ± 0.1 (20)	0.2 ± 0.1 (20)	13 ± 6 (20)	7 ± 2 (20)
	*/NS	NS	NS	NS

[a]During the Control trial, wolves were confined to metabolism cages; during the Exercise trial wolves were exercised daily on a treadmill. Data are given as mean ± standard deviation (sample size). * indicates significant difference at P = 0.05 (ANOVA); NS represents lack of significance at P = 0.05; */NS indicates mixed statistical comparisons. For sodium concentration, 1 of 4 wolves had significant difference.

TABLE 3.11. Blood, urine and fecal nitrogen of captive wolves confined to cages or exercised daily[a] during winter.

Trial	Concentrations		
	Blood (BUN)[b] ($mg \cdot dl^{-1}$)	Urine ($mg \cdot ml^{-1}$)	Feces (% of dry fecal weight)
Control	18.8 ± 2.3 (20)	51.4 ± 4.0 (16)	2.7 ± 0.2 (12)
Exercise	24.2 ± 4.2 (20)	43.5 ± 6.9 (20)	3.2 ± 0.4 (20)
	*	*	*

Trial	Total Daily Losses	
	Urine ($mg \cdot kg^{-1} \cdot d^{-1}$)	Feces ($mg \cdot kg^{-1} \cdot d^{-1}$)
Control	895 ± 116 (16)	114 ± 20 (12)
Exercise	811 ± 155 (20)	122 ± 20 (20)
	NS	NS

[a]During the Control trial, wolves were confined to metabolism cages; during the Exercise trial, wolves were exercised daily on a treadmill. Data are given as mean ± standard deviation (sample size). [b]BUN = blood urea nitrogen concentration. * indicates significant difference at $P = 0.05$ (ANOVA); NS represents lack of significance at $P = 0.05$.

fecal concentration from the control trial to the exercise trial (Table 3.11). Mean total daily urine and fecal losses did not differ between trials.

Gross Energy

Intake

Gross energy content of the dried food was 6.43 ± 0.06 (n = 2) kcal · g^{-1}. Daily gross energy intakes were 104 kcal · kg^{-1} during both trials.

Output

Mean urine energy concentration declined significantly from the control trial to the exercise trial while mean fecal energy concentration remained unchanged (Table 3.12). Mean total daily urine energy loss did not change between trials, but mean total daily fecal energy loss decreased significantly from the control trial to the exercise trial.

Mean daily energy expenditure did not differ between trials (Table 3.12).

Electrolyte, Nitrogen and Energy Balances

There were no significant differences in sodium, potassium, nitrogen or energy balance between trials (Table 3.13).

Blood Glucose and Lipid Concentrations

Mean serum glucose concentration declined significantly from the control trial to the exercise trial in two wolves but did not change in the other two wolves (Table 3.14). Mean serum triglyceride concentration did

TABLE 3.12. Energy parameters of captive wolves confined to cages or exercised daily[a] during winter.

Trial	Energy Expenditure ($kcal \cdot kg^{-1} \cdot d^{-1}$)	Concentrations		Total Daily Losses	
		Urine ($kcal \cdot ml^{-1}$)	Fecal ($kcal \cdot g\ dry\ wt^{-1}$)	Urine ($kcal \cdot kg^{-1} \cdot d^{-1}$)	Fecal ($kcal \cdot kg^{-1} \cdot d^{-1}$)
Control	115 ± 26 (4)	0.26 ± 0.02 (16)	4.6 ± 0.6 (16)	4.8 ± 0.2 (4)	21.7 ± 2.1(4)
Exercise	111 ± 6 (4)	0.22 ± 0.04 (20)	4.1 ± 0.8 (20)	4.9 ± 0.3 (4)	18.0 ± 1.4 (4)
	NS	*	NS	NS	*

[a]During the Control trial, wolves were confined to metabolism cages; during the Exercise trial, wolves were exercised daily on a treadmill. Data are given as mean ± standard deviation (sample size). * indicates significant difference at P = 0.05 (ANOVA); NS represents lack of significance at P = 0.05.

TABLE 3.13. Electrolyte, nitrogen and energy balances of captive wolves confined to cages or exercised daily[a] during winter.

Trial	Balance			
	Sodium ($mg \cdot kg^{-1} \cdot d^{-1}$)	Potassium ($mg \cdot kg^{-1} \cdot d^{-1}$)	Nitrogen ($mg \cdot kg^{-1} \cdot d^{-1}$)	Energy ($kcal \cdot kg^{-1} \cdot d^{-1}$)
Control	-7.1 ± 11.4 (12)	-25.3 ± 14.7 (12)	-31 ± 100 (12)	-36 ± 25 (4)
Exercise	-9.2 ± 9.7 (20)	-21.3 ± 17.2 (20)	+70 ± 160 (20)	+30 ± 7 (4)
	*/NS	NS	NS	NS

[a]During the Control trial, wolves were confined to metabolism cages; during the Exercise trial, wolves were exercised daily on a treadmill. Data are given as mean ± standard deviation (sample size). * indicates significant difference at P = 0.05 (ANOVA); NS represents lack of significance at P = 0.05; */NS indicates mixed statistical comparisons. For sodium balance, 2 of 4 wolves had significant differences.

TABLE 3.14. Blood glucose, triglyceride and free fatty acid concentrations of captive wolves confined to cages or exercised daily[a] during winter.

Trial	Parameter		
	Serum Glucose ($mg \cdot dl^{-1}$)	Serum Triglycerides ($mg \cdot dl^{-1}$)	Plasma Free Fatty Acids ($meq \cdot l^{-1}$)
Control	144 ± 18 (20)	27 ± 3 (20)	0.8 ± 0.2 (16)
Exercise	133 ± 12 (20)	29 ± 9 (19)	0.6 ± 0.1 (20)
	*/NS	NS	*

[a]During the Control trial, wolves were confined to metabolism cages; during the Exercise trial, wolves were exercised daily on a treadmill. Data are given as mean ± standard deviation (sample size). * indicates significant difference at P = 0.05 (ANOVA); NS represents lack of significance at P = 0.05; */NS indicates mixed statistical comparisons. For serum glucose, 2 of 4 wolves had significant differences.

not change between trials, but mean plasma free fatty acid concentration declined significantly from the control to the exercise trial (Table 3.14).

Total Protein Concentration and Hematocrit

There were no significant differences in mean serum total protein concentration or in mean hematocrit between trials (Table 3.15).

Body Temperature

The two mean body temperatures were identical, 38.1 ± 0.6C (n = 95) for the control trial and 38.1 ± 0.7C (n = 96) for the exercise trial.

Ambient Temperature

The mean ambient temperatures at approximately 7:00 A.M. were -29.5 ± 6.9C (n = 9) for the control trial and -20.4 ± 1.9C (n = 9) for the exercise trial.

TABLE 3.15. Serum total protein concentrations and hematocrits of captive wolves confined to cages or exercised daily[a] during winter.

Trial	Parameter	
	Total Protein ($g \cdot dl^{-1}$)	Hematocrit (%)
Control	6.4 ± 0.5 (20)	47 ± 2 (20)
Exercise	6.3 ± 0.5 (20)	47 ± 1 (20)
	NS	NS

[a]During the Control trial, wolves were confined to metabolism cages; during the Exercise trial, wolves were exercised daily on a treadmill. Data are given as mean ± standard deviation (sample size). NS represents lack of significance at P = 0.05 (ANOVA).

DISCUSSION

Water Metabolism and Energy Conservation

Energy Balance

Energy balance was either unchanged between trials or slightly less during the exercise trial. Mean calculated daily energy balance did not differ between trials (Table 3.13). The lack of change in energy balance is supported by the lack of change in mean energy expenditure (Table 3.12) or in mean nitrogen balance (Table 3.13) between trials. Conversely, body weight declined (0.2% $\cdot$ d^{-1}, 48 g $\cdot$ d^{-1} in a 40 kg wolf) during the exercise trial, suggesting lower energy balance than during the control trial. There was a greater tendency for wolves to dehydrate during the exercise trial (Table 3.16), but that water loss accounted for less than half the daily weight loss. Thus, if a decline in energy balance occurred, it was too small to be detected with the sample sizes used for balances and energy expenditure.

The similarity in mean trial energy balances indicated that one or more avenues of energy loss had to be reduced during the exercise trial to compensate for the energy expended during treadmill exercise. It will be argued in the next section that snow intake suppression was not a means to reduce energy expenditure during the exercise trial.

TABLE 3.16. Changes in body composition and hydration of captive wolves confined to cages or exercised daily[a] during winter.

Wolf	Trial	Body Composition Changes					Hydration Changes	
		Δ BW[b] (kg)	Δ Solids[c] (kg)	Δ F[d] (kg)	Δ P[e] (kg)	Δ O[f] (kg)	ΔTBW[g] / Δ Lean ($l \cdot kg^{-1}$)	ΔTBW / Δ P ($l \cdot kg^{-1}$)
Moonlite	Control	+ 0.8	- 0.5	- 0.9	0.0	+ 0.4	0.737 NC	123 ↑
Nanook	Control	+ 0.9	+ 0.2	- 0.2	0.0	+ 0.4	0.727 NC	- 80 ↑
Siqiniq	Control	0.0	- 1.4	- 1.9	0.0	+ 0.5	0.734 NC	46 ↑
Uncle Greg	Control	+ 0.4	- 1.3	- 1.8	- 0.3	+ 0.8	0.744 ↑	- 6 ↑
Moonlite	Exercise	- 0.3	- 1.1	- 1.3	+ 0.1	+ 0.1	0.736 ↑	5.8 ↑
Nanook	Exercise	- 1.0	- 1.2	- 1.3	+ 0.2	- 0.1	0.765 ↑	1.7 ↓
Siqiniq	Exercise	- 1.3	- 1.5	- 1.5	+ 0.1	- 0.1	0.727 NC	1.2 ↓
Uncle Greg	Exercise	- 0.8	- 1.0	- 0.9	+ 0.2	- 0.3	1.222 ↑	0.5 ↓

[a]During the Control trial, wolves were confined to metabolism cages; during the Exercise trial, wolves were exercised on a treadmill daily. [b]Δ BW = change in body weight; [c]Δ Solids = change in body solids; [d]Δ F = change in body fat; [e]Δ P = change in body protein; [f]Δ O = change in body nonfat nonprotein solids. [g]Δ TBW = change in total body water; Δ Lean = change in lean body weight. Control and Exercise data represent changes from beginning to end of trials. Arrows (↑, ↓) indicate direction of change in lean tissue hydration. NC indicates no hydration change; ΔTBW/ΔLean values between 0.727 and 0.737 were considered to be NC.

Water Metabolism

The overall changes in water metabolism associated with daily treadmill exercise did not contribute to energy conservation. In fact, the changes in water intakes and outputs contributed to increased energy loss.

Mean daily evaporative water loss (EWL) was greater during the exercise trial than during the control trial (Table 3.1). Laboratory dogs had greater EWL when running than when resting (O'Connor 1975). Similarly, the greater daily EWL by the wolves was attributed to thermoregulatory EWL during exercise. The marked increase in EWL during and immediately after exercising was plainly visible: the exhaled water vapor condensed and coated the fur with frost.

Urinary and fecal water losses were enhanced in the exercise trial compared to the control trial, and the mean daily fecal water loss had an absolute increase as well. The enhanced urinary water loss was seen as a decrease in mean urine osmolality between trials. In contrast, when greyhounds were exercised daily on a treadmill, mean urine osmolality did not differ significantly from the pretraining mean (McKeever *et al.* 1985). The greyhounds trotted at nearly the same speed, at approximately half the incline and for a similar number of days as the wolves, but low ambient temperature and snow consumption were not factors in the greyhound experiment. Enhanced fecal water loss in the wolves was seen as an increase in fecal water content between trials. These two findings represent decreases in urinary and fecal water conservation. In addition there was an absolute increase in mean daily fecal water loss. It is possible that there was a significant increase in absolute urinary water

loss as well but that the increase was too small to be detected with the sample size used.

Mean daily snow intake was greater during the exercise trial than during the control trial. The increased daily snow intake during the exercise trial was attributed in part to replacement of evaporative water loss. Increased water intake accurately replaced the evaporative losses in dogs exercised daily (Greenleaf *et al.* 1976). Part of the increased snow intake was also associated with the enhanced fecal and urinary water losses. A definitive cause and effect relationship could not be established between increased snow intake and enhanced urinary and fecal water losses, but there are two possibilities. One possible relationship is that snow intake overcompensated for EWL, causing secondary water diuresis and increased fecal water loss. Excessive drinking and subsequent water diuresis have been elicited in dogs by extending the time interval between exercise and drinking (O'Connor and Potts 1969) or by increasing the degree of dehydration (Adolph 1943). There have been no reports of excessive fecal water loss with daily exercise in dogs. A second possible relationship is that the enhanced urinary and/or fecal water losses were direct responses to daily exercise. If so, the snow intake increase was secondary to the urine and fecal water loss as well as to the increased EWL. This possibility was not discussed in the literature.

The decreased urine osmolality and increased fecal water content are indirect evidence of enhanced snow intake. Conversely, the greater tendency for dehydration during the exercise trial (Table 3.16) suggests that snow intake was suppressed. More credence was given to urinary and

fecal water content than to change in hydration because the former were direct measurements while the latter resulted from a series of calculations and therefore had more likelihood for error.

Result and Implications of Testing the Hypothesis

The preceding data gave only weak evidence of snow intake suppression: dehydration during the exercise trial relative to the control trial. The evidence of enhanced snow intake was stronger: enhanced water loss by kidneys and gut. These findings refute the hypothesis that during winter, wolves reduce energy expenditure by suppressing snow intake when energy balance is negative.

Since energy balance differed little, if at all, between trials, there had to have been compensation for the energy expenditure of exercising (864 kcal $\cdot$ d^{-1} for a 40 kg wolf, Appendix E). This compensation could have been (1) a decrease in energy loss through avenues other than snow intake suppression and/or (2) an increase in energy assimilation. Potential decreases in daily energy expenditure may have occurred through decreased activity in the metabolism cages or through postural changes (e.g. increased frequency of curling to conserve heat) between exercise periods. Significant changes in body temperature did not occur and therefore did not decrease daily energy expenditure. A potential increase in energy assimilation occurred through increased nutrient absorption in the gut: individual energy digestibilities (difference between ingested and fecal energy, divided by ingested energy) all increased by 1% to 4%. Thus,

wolves may have evolved adaptations to help compensate for the energy loss of exercise when energy balance is negative or marginal.

The Water Transfer Rate-Energy Expenditure Relationship

The direct relationship between WTR and energy expenditure found in some mammalian species (Macfarlane *et al.* 1971, Kennedy and Macfarlane 1971) was not apparent when captive wolves were exercised during winter. Mean WTR increased significantly from the control trial to the exercise trial, while mean energy expenditure did not differ significantly between trials (Table 3.12). As a result, the individual ratios of WTR to energy expenditure were 1.4 to 2.2 times greater during the exercise trial than during the control trial (Table 3.17). Thus, the water transfer rate-energy expenditure relationship was uncoupled when wolves exercised without increased food intake. The uncoupling was attributed to the increased thermoregulatory EWL during the exercise trial.

Water/Electrolyte Metabolism and Work Performance

The key to potentially increased work performance was a nonisotonic expansion of the resting ECF % of Lean. The increase in ECF % of Lean maintained cardiac output during exercise and the lowered ECF osmolality increased the gradient between resting and exercising osmolality.

Changes in Water and Electrolyte Metabolism

The increased ECF % of Lean between trials was due to increased TBW% (Table 3.2) and to a shift in water from the ICF to the ECF. Evidence of this ICF-ECF water shift was the subcompartment changes as percent of

Table 3.17. Individual water transfer rates, energy expenditures and ratios of water transfer rate to energy expenditure of captive wolves confined to cages or exercised daily[a] during winter.

Wolf	Trial	Water Transfer Rate ($ml \cdot kg^{-1} \cdot d^{-1}$)	Energy Turnover ($ml \cdot kg^{-1} \cdot d^{-1}$)	Water Transfer Rate / Energy Turnover (moles H_2O·moles O_2^{-1})
Moonlite	Control	30.9	100	1.8
Nanook	Control	32.1	88	2.1
Siqiniq	Control	36.5	137	1.6
Uncle Greg	Control	35.8	133	1.6
mean ± s.d.				1.8 ± 0.2 (4)
Moonlite	Exercise	62.5	110	3.3
Nanook	Exercise	56.9	112	3.0
Siqiniq	Exercise	60.0	120	2.9
Uncle Greg	Exercise	61.0	105	3.5
mean ± s.d.				3.2 ± 0.2 (4)

[a]During the Control trial, wolves were confined to metabolism cages; during the Exercise trial, wolves were exercised on a treadmill daily.

lean body weight (Tables 3.4 and 3.5). Extracellular fluid content of the lean body increased in all four wolves while intracellular fluid content of the lean body declined. The increase in ECF % of Lean occurred predominantly in the ISF space rather than in the PV space: the changes in PV % of Lean were mixed and of much smaller volumes than the changes in ISF% of Lean (Tables 3.4 and 3.7).

The fluid shift from ICF to ECF was ascribed primarily to a net shift in potassium from the ICF to the ECF. The evidence of this potassium shift is the increase of 21% to 65% in calculated total ECF potassium (ECF volume • serum potassium concentration) between the end of the control trial and the end of the exercise trial (Table 3.18). It is also possible that the decline in intracellular water at the end of the exercise trial was due in part to a depletion of glycogen stores during the exercise trial. No measurements of glycogen levels were made in either trial, however.

The decline in mean serum sodium concentration from the control trial to the exercise trial (Table 3.8) was the primary determinant of the decline in serum (ECF) osmolality. There was a tendency for glucose concentration to decline between trials (Table 3.14), but glucose accounts for less than 7% of the serum osmolality (Grauer and Grauer 1983). The only other molecules which contribute significantly of serum osmolality are potassium and urea. Neither potassium nor urea nitrogen concentration declined between trials (Tables 3.8 and 3.11). The decreased sodium concentration between trials did not result from increased serum total proteins and/or serum lipids. There were no increases in serum total protein (Table 3.15), triglyceride or free fatty acid (Table 3.14)

TABLE 3.18. Total extracellular potassium of captive wolves confined to cages or exercised daily[a] during winter.

Wolf	Total ECF[b] Potassium at end of Control (meq)	Total ECF Potassium at end of Exercise (meq)	Change in Total ECF Potassium (meq)
Moonlite	40	52	+ 30%
Nanook	31	51	+ 65%
Siqiniq	35	45	+ 29%
Uncle Greg	39	47	+ 21%

[a]During the Control trial, wolves were confined to metabolism cages; during the Exercise trial, wolves were exercised on a treadmill daily. [b]ECF = extracellular fluid.

concentrations between trials. The next section discusses how the changes in water and electrolyte metabolism could improve work performance.

Improved Work Performance

The lower resting serum osmolality associated with daily exercise would maintain work performance through a greater degree of water loss than without daily exercise. Acute dehydration (O'Connor 1975) with increased serum sodium concentration and osmolality (Greenleaf *et al.* 1976) has been documented in running dogs. It has been shown that dehydration is deleterious to human work performance, and the magnitude of effect increases with increased dehydration (Craig and Cummings 1965, Strydom *et al.* 1968). Furthermore, central nervous system abnormalities occur at serum osmolalities of 340 mOsm·kg^{-1} and higher (Green 1978). Thus, it is reasoned that by lowering resting serum osmolality, wolves increase the degree of dehydration which they can sustain during exercise before high serum osmolality decreases work performance.

A possible mechanism for the decreased resting serum osmolality was a training-induced decline in the osmolality set point. This change would occur in the hypothalamus. It is hypothesized that during training, vasopressin (ADH) activity was enhanced and/or thirst was increased until the new set point was reached. A future study should address this hypothesis.

The greater ISF associated with daily exercise would enhance work performance by maintaining PV. After eight to ten days of continuous

exercise training (cross-country skiing), humans had increased resting PVs (Holmgren *et al.* 1964). Presumably, increased resting PV increased the degree of dehydration sustainable before work performance declined. Plasma volume was a primary determinant of cardiac output, and cardiac output was considered a primary determinant of work performance. It is suggested that increased resting ISF in the wolves acted as a reservoir to maintain PV and therefore cardiac output and work performance during exercise.

In the wild, maintenance of work performance would increase the efficiency of chasing and killing prey. Adequate water intake was found to be the single most important factor in maintaining work performance (Pitts *et al.* 1944, Adolph *et al.* 1947, Young *et al.* 1959) for humans and dogs. Minimizing stops to consume snow would improve efficiency of the wolves in hunting, chasing and killing prey.

CONCLUSIONS

1. When daily exercise without food increase was imposed on captive wolves during winter, energy balance had little or no difference from control levels. Energy was conserved by increased efficiency of absorption from the gut and by other undocumented processes, but not by alteration of water metabolism. There was indirect evidence of both suppressed and enhanced snow intake, although the evidence of enhancement was more definitive. These findings refute the hypothesis that during winter, wolves reduce energy expenditure by suppressing snow intake when energy balance is negative.

2. There was not a direct relationship between water transfer rate and energy expenditure when wolves were either confined to cages or exercised daily with no change in food intake. Uncoupling of the water transfer rate-energy expenditure relationship was due to the increase in daily evaporative water loss and little or no change in total daily energy expenditure with daily exercise.

3. Daily exercise in winter was associated with increased extracellular water and decreased extracellular fluid osmolality in captive wolves. These two adaptations could enhance work performance by delaying two deleterious effects of acute dehydration: decline in cardiac output and increase in extracellular fluid osmolality. It is hypothesized that these two changes occurred during training, through increased thirst and/or increased vasopressin activity.

GENERAL SUMMARY AND CONCLUSIONS

It was hypothesized that during negative energy balance in winter, wolves reduce energy expenditure by suppressing snow intake. The hypothesis was tested by subjecting captive wolves to various forms of negative energy balance during arctic winter and determining whether snow intake was suppressed. Experiments were designed to impose negative energy balance through (1) undernutrition, (2) fasting, (3) alternate gorging-fasting and (3) forced exercise without a change in food intake.

The goal of this study was to determine whether wolves conserve a significant quantity of energy by suppressing snow intake during negative energy balance in winter. The amount by which snow intake is suppressed can be equated to an energy savings: the quantity of energy required to melt that amount of snow and warm the resulting water to body temperature.

During undernutrition, captive wolves suppressed snow intake, but the amount of energy conserved by doing so was inconsequential. The evidence of snow intake suppression during undernutrition was indirect: dehydration, decreased fecal water content and possibly decreased snow intake as a proportion of total water intake. The estimated maximum total energy saved by snow intake suppression during undernutrition was less than 1% of the calculated daily energy budget. The results of this experiment supported the hypothesis but revealed that the amount of energy saved was inconsequential.

The wolves did not suppress snow intake during fasting. The evidence for lack of snow intake suppression during fasting was indirect: during fasting, urine concentration declined and fecal water content increased, compared to values during gorging and during daily maintenance feeding. These results refuted the hypothesis.

The wolves did not suppress overall snow intake during alternate gorging-fasting. In fact, there was evidence of greater overall snow intake during alternate gorging-fasting than during daily maintenance feeding: during alternate gorging-fasting, overall tissue hydration and the overall snow intake-dry matter intake ratios were greater than during daily maintenance feeding. Despite the lack of snow intake suppression, the hypothesis could not be tested because decreased energy balance during alternate gorging-fasting could not be documented.

Evidence of change in snow intake was equivocal when the wolves were exercised daily without an increase in food intake. There was a tendency for dehydration during the exercise trial, suggesting snow intake suppression. There was, however, stronger evidence of enhanced snow intake during the exercise trial: decreased urine concentration and increased fecal water content. These findings refuted the hypothesis.

It is possible that snow intake was enhanced during exercise training. Total body water content increased from the control trial to the exercise trial. It was hypothesized that the mechanism for increased total body water content was increased thirst and/or increased antidiuretic hormone activity. This hypothesis remains to be tested by a future study.

Overall, experimental results refuted the hypothesis but met the goal of this study. Definitive evidence of suppressed snow intake occurred only during undernutrition. The lack of snow intake suppression during either fasting or the exercise trial was sufficient to refute the hypothesis. Despite the suppression of snow intake during undernutrition, the amount of energy conserved was inconsequential.

Based on the data from this study, snow intake suppression is not a practical means of energy conservation for wolves during winter. In seven of the eight trials, the most energy which could have been saved by totally eliminating snow intake was only 4.3% of the mean daily energy expenditure (see Appendix F). It is therefore concluded that when energy balance is negative during winter, wolves do not suppress snow intake to conserve energy.

APPENDIX A

Body Weights of Captive Wolves at Varying Food Intake and Exercise Levels During Winter

Body weights (kg) of captive wolves at three food intake levels[a] during winter.

		Body Weight						
		Trial Day						
Wolf	Trial	1	2	3	4	5	6	7
Rusty	Undernutrition	37.5	37.0	37.3	37.3	36.6	36.4	36.1
Butch	Undernutrition	40.0	39.3	39.5	39.1	38.9	38.6	38.4
Mix	Undernutrition	35.0	35.0	34.1	33.9	33.4	33.2	33.4
Frosty	Undernutrition	40.5	40.0	40.0	39.3	39.1	39.1	38.9
Mr. Brown	Undernutrition	35.5	35.9	35.0	34.8	34.5	34.5	34.1
		Trial Day						
		8	9	10	11	12	13	14
Rusty	Undernutrition	36.1	35.9	35.9	35.9	35.9	35.7	35.7
Butch	Undernutrition	38.2	38.0	37.7	38.0	38.0	38.0	37.7
Mix	Undernutrition	33.0	32.5	32.5	32.3	32.6	32.3	32.3
Frosty	Undernutrition	38.6	38.4	38.2	38.2	38.4	38.2	38.2
Mr. Brown	Undernutrition	33.4	33.9	33.6	33.4	33.5	33.2	33.4
		Trial Day						
		1	2	3	4	5	6	7
Rusty	Maintenance	35.7	35.9	35.8	36.4	36.3	36.6	36.4
Butch	Maintenance	37.5	37.7	38.3	38.6	38.8	38.9	38.9
Mix	Maintenance	32.3	32.3	32.6	32.3	32.7	32.8	32.7
Frosty	Maintenance	38.1	38.4	38.6	38.9	39.1	39.0	38.9
Mr. Brown	Maintenance	33.2	33.6	34.0	33.9	34.1	34.3	34.1

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		Trial Day 8	9	10	11	12	13	14
Rusty	Maintenance	36.8	36.8	36.9	36.9	37.0	37.4	37.6
Butch	Maintenance	39.1	39.1	39.2	39.0	39.4	39.9	40.0
Mix	Maintenance	32.8	33.0	32.7	33.0	33.1	33.0	33.4
Frosty	Maintenance	39.1	39.1	39.2	39.5	39.1	39.5	39.9
Mr. Brown	Maintenance	34.2	34.7	34.4	34.4	34.5	34.7	35.5

		Trial Day 1	2	3	4	5	6	7
Rusty	Overnutrition	37.5	38.3	38.5	39.2	39.5	39.9	40.7
Butch	Overnutrition	39.8	40.9	40.1	41.5	42.2	42.5	42.8
Mix	Overnutrition	33.4	34.1	34.3	34.3	35.0	35.7	35.5
Frosty	Overnutrition	40.2	40.3	41.6	41.1	42.4	42.5	42.7
Mr. Brown	Overnutrition	35.2	36.0	36.8	36.6	37.3	37.4	38.0

		Trial Day 8	9	10	11	12	13	14
Rusty	Overnutrition	41.3	40.9	41.6	42.6	42.4	42.6	42.7
Butch	Overnutrition	43.5	43.6	44.1	44.4	44.7	44.1	44.5
Mix	Overnutrition	35.9	36.5	36.4	36.5	37.2	37.4	37.5
Frosty	Overnutrition	42.7	43.2	43.2	42.8	43.1	42.8	43.4
Mr. Brown	Overnutrition	38.5	38.9	39.2	39.7	40.1	40.1	40.1

*Wolves were fed Zu/Preem daily at 18.75 $g \cdot kg^{-1}$ during Undernutrition, 37.50 $g \cdot kg^{-1}$ during Maintenance and 75.00 $g \cdot kg^{-1}$ during Overnutrition.

Body weights (kg) of captive wolves fed daily or gorged and fasted[a] during winter.

Wolf	Trial	Body Weight, Trial Day 1	2	3	4	5	6	7
Rusty	Control Trial	42.0	42.4	42.7	42.6	42.6	42.5	43.4
Butch	Control Trial	42.5	42.5	42.5	42.2	42.0	42.6	43.2
Mix	Control Trial	36.4	36.2	36.1	36.4	36.8	36.8	36.8
Frosty	Control Trial	43.4	44.0	44.7	44.6	44.4	44.8	45.6
Mr. Brown	Control Trial	40.8	40.4	40.1	40.4	40.8	40.8	40.7

		Trial Day 1	2	3	4	5	6
Rusty	4-Day Gorge-Fast Trial	41.8	44.0	44.3	43.0	42.4	44.1
Butch	4-Day Gorge-Fast Trial	42.0	41.8	44.5	43.0	42.3	44.1
Mix	4-Day Gorge-Fast Trial	34.1	35.7	35.1	34.5	34.4	35.6
Frosty	4-Day Gorge-Fast Trial	45.0	45.7	46.0	45.7	45.7	46.4
Mr. Brown	4-Day Gorge-Fast Trial	40.7	42.7	42.7	41.8	40.5	43.2

		Trial Day 7	8	9	13	17
Rusty	4-Day Gorge-Fast Trial	44.8	43.6	43.2	43.2	43.5
Butch	4-Day Gorge-Fast Trial	45.2	43.5	42.7	43.3	43.9
Mix	4-Day Gorge-Fast Trial	35.9	34.8	34.4	34.3	34.4
Frosty	4-Day Gorge-Fast Trial	46.9	46.0	45.6	44.8	44.0
Mr. Brown	4-Day Gorge-Fast Trial	43.6	41.6	41.1	41.5	41.1

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		Trial Day 1	3	5	7	9	17
Rusty	8-Day Gorge-Fast Trial	40.8	43.6	44.1	42.5	41.1	41.4
Butch	8-Day Gorge-Fast Trial	39.4	43.0	43.2	41.1	39.7	40.0
Mix	8-Day Gorge-Fast Trial	31.7	35.1	33.9	32.5	31.8	32.0
Frosty	8-Day Gorge-Fast Trial	43.0	44.5	44.5	43.0	42.2	41.3
Mr. Brown	8-Day Gorge-Fast Trial	40.3	42.8	43.6	41.1	38.2	40.0

[a]During the control trial, wolves were fed a weight-maintaining diet daily. During the 4-Day Gorge-Fast trial, wolves were fed a four-day ration in two days and fasted for two days. During the 8-Day Gorge-Fast trial, wolves were fed an eight-day ration in four days and fasted for four days.

Body weights (kg) of captive wolves confined to cages or exercised daily[a] during winter.

Wolf	Trial	Body Weight, Trial Day 1	3	5	7	8
Moonlite	Control Trial	50.7	50.8	51.6	51.4	51.5
Nanook	Control Trial	46.8	47.0	48.0	47.7	47.7
Siqiniq	Control Trial	40.9	40.9	41.0	40.8	40.9
Uncle Greg	Control Trial	46.8	47.4	47.5	47.5	47.2
		Trial Day 1	3	5	7	9
Moonlite	Exercise Trial	51.1	51.0	51.4	50.9	50.8
Nanook	Exercise Trial	50.2	49.3	49.7	50.0	49.6
Siqiniq	Exercise Trial	43.8	43.2	43.4	42.6	42.6
Uncle Greg	Exercise Trial	45.8	45.3	45.6	45.0	45.0

[a]During the control trial, wolves were confined to metabolism cages. During the exercise trial, wolves were exercised daily on a treadmill.

APPENDIX B

Calculation of Water Intake and Output Using Equations for Nonsteady State

A nonsteady state existed at the beginning of each food intake trial (Chapter 1). Therefore, water output (WO, ml $\cdot$ kg^{-1} $\cdot$ d^{-1}) and intake (WI, ml $\cdot$ kg^{-1} $\cdot$ d^{-1}) rates were calculated according to equations derived for subjects in which total body water (TBW) was changing with rapid growth (Holleman *et al.* 1982b):

$$WO = (TBW_2 - TBW_1) \cdot \ln(TBW_1 \cdot S_1 \cdot TBW_2^{-1} \cdot S_2^{-1}) \cdot (t_2 - t_1) \cdot [\ln(TBW_2 \cdot TBW_1^{-1})]^{-1}$$

$$WI = WO + (TBW_2 - TBW_1) \cdot (t_2 - t_1),$$

where S is specific activity (DPM $\cdot$ ml^{-1}), t is time (days) and the subscripts 1 and 2 represent initial and final values, respectively.

The resulting WOs and WIs are of limited value because the conditions of the food intake trials did not match those for which the above equations were derived. The equations were derived for rapidly growing animals in which TBW varied linearly with time. It is not possible to ascertain from the Chapter 1 data whether the wolf TBWs varied linearly with time. Since the accuracy of WO and WI calculation is questionable, water transfer rate was used for all comparisons and linear regressions. The WO and WI data are included for comparison.

Water intake and output of captive wolves at three food intake levels[a].

Parameter ($ml \cdot kg^{-1} \cdot d^{-1}$)	Food Intake Trial		
	Undernutrition	Maintenance	Overnutrition
Water Intake (WI)	26.1 ± 6.6 (4)	39.5 ± 6.4 (5)	62.5 ± 10.2 (5)
Water Output (WO)	28.0 ± 6.3 (4)	37.5 ± 6.8 (5)	61.3 ± 9.7 (5)

[a]Undernutrition food intake = 18.75 $g \cdot kg^{-1} \cdot d^{-1}$; Maintenance food intake = 37.50 $g \cdot kg^{-1} \cdot d^{-1}$; Overnutrition food intake = 75.00 $g \cdot kg^{-1} \cdot d^{-1}$. Each trial lasted 14 days. Data are given as mean ± standard deviation (sample size).

APPENDIX C

Comments On Radioisotope Techniques

Tritiated Water

The tritiated water dilution method is commonly used to estimate total body water *in vivo*. When this method is used there are five assumptions which, if not met, can lead to errors (Nagy and Costa 1980, Holleman *et al.* 1982b).

The first assumption is that true TBW, water intake and water output were constant over the time of injection and sample collection. It is likely that this assumption was met when blood samples were collected from the wolves within 4.5 hours of isotope injection. This assurance is based partly on food and water deprivation for four to twelve hours before and 4.5 hours after isotope injection. In addition, most of the 24 hour urination and defecation occurred in the morning, prior to isotope injection and blood sampling, and exercise occurred after injection and sampling. When sampling was done over fourteen days (Chapter 1), the extrapolated DPM at t_0 differed from DPM within the first 4.5 hours by only 0.6 $\pm$ 1.8%. This small percent difference suggested that water intake and output were relatively constant in the food intake and exercise experiments (Chapters 1 and 3); such an evaluation was not made for the gorge-fast trials.

The second assumption is that TBW was a single compartment in which the tritiated water was distributed rapidly and uniformly. In this study, the straight lines formed by plots of specific activity against time

(Chapter 1) had correlation coefficients greater than 0.996. The data from the exercise experiment (Chapter 3) were not evaluated in this manner but were assumed to produce similar coefficients. Such lines are characteristic of a one-compartment steady state open system (Holleman *et al.* 1982b). Although the gorge-fast data (Chapter 2) cannot be assumed to produce such coefficients, intravenous injection of tritiated water assured the most rapid distribution possible under the experimental conditions. These conditions (Nagy and Costa 1980) - e.g. lack of severe dehydration, use of a carnivore instead of a ruminant - also favored rapid distribution. Uniform distribution can be safely assumed (Holleman *et al.* 1982b).

The third assumption is that tritium did not exchange with nonwater body constituents and was only eliminated as water. This assumption is not met in tritiated water studies (Nagy and Costa 1980, Holleman *et al.* 1982b). As a consequence, calculated compartment sizes overestimate direct measurements by up to 13.8% (Olsson 1970, Sheng and Huggins 1971, Tisavipat *et al.* 1974, Holleman and Dieterich 1975, Culebras *et al.* 1977, Sato *et al.* 1979, Nagy and Costa 1980). In one compilation of data from validation studies on terrestrial mammals, most estimates by tritiated water differed from direct measurements by +1.6% to +6.5% (Nagy and Costa 1980). Thus it is assumed that the calculated wolf TBWs are within 1.6% to 6.5% of the true TBW, and no further corrections were made.

The fourth assumption is that isotopic water concentration was the same in TBW and in water lost from the body. This assumption might not

have been met for water lost during ventilation. Heavy water evaporates more slowly than regular water, resulting in underestimation of TBW and fractional turnover rate. Errors due to such physical fractionation are generally inconsequential (Holleman *et al.* 1982b).

The last assumption is that no water entered the body through lungs or skin during the measurement period. Exchange of tritiated water for nonisotopic inhaled or contacted water would lead to overestimation of TBW and fractional turnover rate. No appreciable error was attributed to this assumption in the present study because no water contacted the skin and the water content of ambient air was inconsequential. The water vapor capacity of cold air is less than that of warm air, and most ambient air temperatures during this study were between -12C and -40C. The vapor pressures of saturated air at -12C and -40C are approximately 10% and 0.6%, respectively, of the vapor pressure of saturated air at 20C (Weast 1970).

Radiosulfate

Radiosulfate has been used to measure the functional extracellular fluid space rather than total ECF (i.e. all the body water outside the cells) (Walser *et al.* 1953, Middleton *et al.* 1969). The two major subdivisions excluded from total ECF in measuring functional ECF are: (1) ECF compartments which might be considered outside of the body, such as the gastrointestinal tract and lumina of glands; and (2) "non-functional" ECF, such as connective tissue water and organic sites which bind radiosulfate. These compartments are not in diffusion equilibrium with

the rest of the ECF and thus act like intracellular water (Walser *et al.* 1953, Middleton *et al.* 1969).

There are two general methods of processing blood samples for counting radiosulfate. In one, radiosulfate is oxidized to magnesium sulfate (Jeffay *et al.* 1960) or precipitated as the benzidine salt (Walser *et al.* 1953, Walser 1967, Barrett and Walser 1969) so that only the inorganic radiosulfate is counted. In the other method, plasma is mixed with 10% or 20% trichloroacetic acid (TCA) and the protein-free supernatant containing both inorganic and organic radiosulfate is counted (Walser *et al.* 1953, Bauer *et al.* 1975a, Bauer *et al.* 1975b). The latter method is simpler, and the failure to include organic radiosulfate has had no appreciable effect on functional ECF measurements in man and dogs (Walser *et al.* 1953).

In most radiotracer studies, extrapolation of specific activity to zero time has been used for calculation of body water spaces (Bauer *et al.* 1975b). For calculations, this procedure eliminates collecting and counting urine samples. The results are reproducible, and although they are not necessarily absolute measurements they are acceptable for comparative studies.

Plasma Volume

The use of radioiodinated (^{125}I) human serum albumin (RIHSA) in the dog has been evaluated by comparison to radioiodinated (^{131}I) dog serum albumin (RIDSA) (Hood and Hightower 1976). The two isotopes compared favorably with each other and with previous findings although RIHSA

tended to slightly overestimate PV. Thus the choice of RIHSA was acceptable for the wolf study. Radioiodinated human serum albumin was chosen over radioiodinated dog or bovine serum albumin because of availability and convenience.

Extrapolation of specific activity to time zero has introduced error into PV determinations (Swan and Nelson 1971, Bauer *et al.* 1975b). The errors resulted from the constant change in blood RIHSA concentration and variability in the rate of that change (Swan and Nelson 1971). Such changes occurred in the wolf study: the slopes of the extrapolation lines for wolves varied considerably, with some positive, some negative and some near zero. Mean CPM from blood samples at t_0+30, +45 and +60 minutes in humans and dogs has given better than 70% accuracy in detecting PV changes, and equilibration was reached after five minutes (Bauer *et al.* 1975b). Therefore the mean CPM from t_0+15, +20 and +25 minutes was used in the wolf study.

Simultaneous Use of Hydrogen-3, Sulfur-35 and Iodine-125

Three body water compartments were estimated simultaneously in Chapters 2 and 3. Total body water was estimated with tritiated water (^3H_2O, TOH), extracellular fluid with radiosulfate ($Na_2{}^{35}SO_4$) and plasma volume with radioiodinated (^{125}I) human serum albumin. The simultaneous use of these three isotopes in dogs and humans has been reported previously (Bauer *et al.* 1975b). In dogs, variations between two sets of measurements taken a week apart were 8%, 8% and 10% for TBW,

ECF and PV, respectively, and part of the difference was attributed to a 2.2% increase in body weight. These variabilities were judged acceptable.

Precipitation of plasma protein with TCA adequately removed the ^{125}I, and the use of windows for simultaneous tritium and radiosulfate counting has been found to be reliable (Bauer *et al.* 1975a, Bauer *et al.* 1975b). After precipitation of plasma proteins, there was not sufficient gamma activity in the supernatant to register above background in the beta channels. Furthermore, a supernatant containing TOH and radiosulfate in concentrations approximating those in the wolf samples did not register above background in a gamma counter set to count ^{125}I.

APPENDIX D

Calculation of Total Daily Energy Expenditure and Comparisons Among Trials and Experiments

Total daily energy expenditure[a] (kcal·kg^{-1}) of captive wolves at three food intake levels[b] during winter.

Wolf	Trial	Energy Intake	Fecal Energy	Urinary Energy	Metabolizable Energy	Energy From Body Tissues	Total Energy Expenditure
Rusty	Undernutrition	52.0	12.0	2.2	37.8		
Butch	Undernutrition	52.0	15.3	2.9	33.8	13.0	46.8
Mix	Undernutrition	52.0	12.7	3.1	36.2	21.9	58.1
Frosty	Undernutrition	52.0	9.3	3.1	39.6	27.5	67.1
Mr. Brown	Undernutrition	52.0	9.2	3.1	39.7	25.4	65.1
mean ± s.d.							59.3 ± 9.2
Rusty	Maintenance	104.0	14.8	4.2	85.0	- 9.9	75.1
Butch	Maintenance	104.0	18.5	4.6	80.9	- 39.3	41.6
Mix	Maintenance	104.0	17.6	4.9	81.5	0.1	81.6
Frosty	Maintenance	104.0	12.7	4.0	87.3	25.0	112.3
Mr. Brown	Maintenance	104.0	17.7	3.8	82.5	- 34.8	47.7
mean ± s.d.							71.7 ± 28.5
Rusty	Overnutrition	208.0	31.3	7.1	169.6	- 59.9	109.7
Butch	Overnutrition	208.0	27.8	6.2	174.0	- 48.3	125.7
Mix	Overnutrition	208.0	31.9	6.6	169.5	- 79.7	89.8
Frosty	Overnutrition	208.0	16.6	4.6	186.8	- 83.9	102.9
Mr. Brown	Overnutrition	208.0	32.4	6.8	168.8	- 84.5	84.3
mean ± s.d.							102.5 ± 16.5

[a]Metabolizable energy = energy intake - fecal energy - urinary energy. Total energy expenditure = metabolizable energy + energy from body tissues. Energy balance = - (energy from body tissues).

[b]Wolves were fed Zu/Preem daily at 18.75 g·kg^{-1} during Undernutrition, 37.50 g·kg^{-1} during Maintenance and 75.00 g·kg^{-1} during Overnutrition.

Total daily energy expenditure[a] ($kcal \cdot kg^{-1}$) of captive wolves fed daily or gorged and fasted[b] during winter.

Wolf	Trial	Average Energy Intake	Fecal Energy	Urinary Energy	Metabolizable Energy	Energy From Body Tissues	Total Energy Expenditure
Rusty	Control	104.0	20.9	4.4	78.7	- 56.1	22.6
Butch	Control	104.0	20.5	4.3	79.2	- 68.5	10.7
Mix	Control	104.0	21.5	4.2	78.3	- 29.2	49.1
Frosty	Control	104.0	-	-	-	-	-
Mr. Brown	Control	104.0	21.9	4.9	77.2	- 46.1	31.1
mean ± s.d.							19.8 ± 23.8
Rusty	4-Day Gorge-Fast	104.0	18.3	3.2	82.5	- 29.0	53.5
Butch	4-Day Gorge-Fast	104.0	18.8	3.5	81.7	- 36.7	45.0
Mix	4-Day Gorge-Fast	104.0	19.8	3.9	80.3	- 8.4	71.9
Frosty	4-Day Gorge-Fast	104.0	15.9	3.3	84.8	12.2	97.0
Mr. Brown	4-Day Gorge-Fast	104.0	19.8	3.7	80.5	- 11.2	69.3
mean ± s.d.							67.3 ± 20.0
Rusty	8-Day Gorge-Fast	104.0	23.4	4.6	76.0	- 4.3	71.7
Butch	8-Day Gorge-Fast	104.0	-	-	-	-	-
Mix	8-Day Gorge-Fast	104.0	17.9	3.6	82.5	- 12.3	70.2
Frosty	8-Day Gorge-Fast	104.0	11.9	2.9	89.2	- 1.3	87.9
Mr. Brown	8-Day Gorge-Fast	104.0	15.4	3.2	85.4	- 11.1	74.3
mean ± s.d.							76.0 ± 8.1

[a]Metabolizable energy = energy intake - fecal energy - urinary energy. Total energy expenditure = metabolizable energy + energy from body tissues. Energy balance = - (energy from body tissues).
[b]During the control trial, wolves were fed a weight-maintaining diet daily. During the 4-Day Gorge-Fast trial, wolves were fed a four-day ration in two days and fasted for two days. During the 8-Day Gorge-Fast trial, wolves were fed an eight-day ration in four days and fasted for four days.

Total daily energy expenditure[a] (kcal·kg^{-1}) of captive wolves confined to cages or exercised daily[b] during winter.

Wolf	Trial	Energy Intake	Fecal Energy	Urinary Energy	Metabolizable Energy	Energy From Body Tissues	Total Energy Expenditure
Moonlite	Control	104.0	23.2	4.0	76.8	22.7	99.5
Nanook	Control	104.0	18.7	3.5	81.8	5.8	87.6
Siqiniq	Control	104.0	23.1	4.8	76.1	61.3	137.4
Uncle Greg	Control	104.0	21.8	4.4	77.8	55.0	132.8
mean ± s.d.							114.3 ± 24.5
Moonlite	Exercise	104.0	20.1	5.4	78.5	32.0	110.5
Nanook	Exercise	104.0	17.4	4.2	82.4	30.0	112.4
Siqiniq	Exercise	104.0	17.9	4.4	81.7	37.8	119.5
Uncle Greg	Exercise	104.0	16.8	3.8	83.4	21.2	104.6
mean ± s.d.							111.8 ± 6.1

[a]Metabolizable energy = energy intake - fecal energy - urinary energy. Total energy expenditure = metabolizable energy + energy from body tissues. Energy balance = - (energy from body tissues).
[b]During the control trial, wolves were confined to metabolism cages. During the exercise trial, wolves were exercised daily on a treadmill.

Several of the above mean energy expenditures were higher or lower than expected. The high values occurred during Overnutrition, the exercise trial and the exercise control trial. Mech (1970) reports the estimated daily expenditure of a 40 kg dog to be 2800 kcal or 70 kcal · kg^{-1}. The daily energy expenditures in the three trials above were 102, 112 and 114 kcal · kg^{-1}, respectively. There are two possible explanations for the difference between the values for dogs and wolves. One explanation is that the wolf values are accurate. In that case, the energy expenditure

above basal metabolism was equivalent to approximately four times the energy expended in 1.25 hours on the treadmill. Such a quantity is unlikely but possible. The other possibility is error in the calculation. The most likely source of error is in calculation of energy derived from body tissues. The errors in these calculations result from extrapolating change in body fat and body protein from measurements of total body water and nitrogen balance. The unreasonably low energy expenditure was 20 kcal · kg^{-1} for the gorge-fast control trial. This value is less than the basal metabolic rate and could only result from errors in extrapolating. These inconsistencies in daily energy expenditure must remain unresolved until accurate daily energy expenditure measurements are made for wolves under the same conditions.

APPENDIX E

Calculation of Energy Expenditure for Wolves Running on a Treadmill

The energy expended during treadmill exercise by wolves had horizontal and vertical components because the treadmill was positioned at a 10% incline. The horizontal energy expenditure component was estimated from the equation of Taylor (1973):

$VO_2 = (8.46\ W^{-0.40})(VEL) + 6.0\ W^{-0.25}$, where

VO_2 = oxygen consumption in ml $O_2 \cdot g^{-1} \cdot h^{-1}$

W = body weight in g

VEL = running velocity in km $\cdot h^{-1}$.

The vertical component of energy expenditure was estimated from the oxygen cost of ascent as determined by White and Yousef (1978) for reindeer walking uphill on pavement at a 9% incline:

1.51 $mlO_2 \cdot g^{-1} \cdot$ vertical km^{-1}.

Oxygen consumption was converted to kcal $\cdot g^{-1} \cdot h^{-1}$ with the factor 4.69 kcal $\cdot$ 10_2^{-1} (Brody 1945), assuming a respiratory quotient of 0.71 for primarily fat catabolism during running.

The total energy expenditure of treadmill running was calculated by adding the energy cost of horizontal and vertical locomotion and subtracting the estimated energy expenditure due to basal metabolism. The energy expenditure of basal metabolism was estimated from the following equation (Prosser 1973):

$M = 70 \cdot W^{0.74}$, where

M = basal metabolism in kcal $\cdot$ d^{-1}

W = body weight in kg.

Only the proportion of basal metabolism during the 1.25 h of running was subtracted from the sum of the horizontal and vertical energy expenditure components.

For a 40 kg wolf, the daily energy expenditure during treadmill running was estimated as follows:

horizontal component	= +463 kcal
vertical component	= +460 kcal
basal metabolism	= - 59 kcal
total	864 kcal.

APPENDIX F

Calculated Energy Cost of Snow Consumption

Energy cost of snow consumption[a] by captive wolves at three food intake levels[b] during winter.

Trial	Mean Daily Energy Expenditure (kcal·kg^{-1})	Cost of Snow Consumption (kcal·kg^{-1})	Cost of Snow Consumption (% of Daily Expenditure)
Undernutrition	59	1.0	1.7
Maintenance	72	1.7	2.3
Overnutrition	102	3.0	2.9

[a]Cost of snow consumption was calculated for snow temperature = -40C and body temperature = 37C. [b]Wolves were fed Zu/Preem daily at 18.75 g·kg^{-1} during Undernutrition, 37.50 g·kg^{-1} during Maintenance and 75.00 g·kg^{-1} during Overnutrition.

Energy cost of snow consumption[a] by captive wolves fed daily or gorged and fasted[b] during winter.

Trial	Mean Daily Energy Expenditure (kcal·kg^{-1})	Cost of Snow Consumption (kcal·kg^{-1})	Cost of Snow Consumption (% of Daily Expenditure)
Control	20	2.4	11.9
4-Day Gorge-Fast	67	2.9	4.3
8-Day Gorge-Fast	75	2.0	2.6

[a]Cost of snow consumption was calculated for snow temperature = -40C and body temperature = 37C. [b]During the Control trial, wolves were fed a weight-maintaining diet daily. During the 4-Day Gorge-Fast trial, wolves were fed a four-day ration in two days and fasted for two days. During the 8-Day Gorge-Fast trial, wolves were fed an eight-day ration in four days and fasted for four days.

Energy cost of snow consumption[a] by captive wolves confined to cages or exercised daily[b] during winter.

Trial	Mean Daily Energy Expenditure ($kcal \cdot kg^{-1}$)	Cost of Snow Consumption ($kcal \cdot kg^{-1}$)	Cost of Snow Consumption (% of Daily Expenditure)
Control	114	1.2	1.1
Exercise	112	3.7	3.3

[a]Cost of snow consumption was calculated for snow temperature = -40C and body temperature = 37C. [b]During the Control trial, wolves were confined to metabolism cages. During the Exercise trial, wolves were exercised daily on a treadmill.

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